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ACIDITY OF SILAGE MADE FROM VARIOUS CROPS[1]

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INTRODUCTION

It has been found that the quality of corn silage is chiefly dependent upon the kind of acids formed during the fermentation (*3, 4, 10*).[2] The purpose of this investigation is to ascertain whether the same acids are developed when other commonly grown crops are used for silage purposes. Corn (*Zea mays*) is the leading crop grown for silage, owing to the heavy yield of green material obtained per acre, but in many sections of the Pacific Northwest the growing of corn is prohibitive because of variable climatic conditions caused by different altitudes. In these sections, when silage is desired, crops other than corn must necessarily be grown.

REVIEW OF LITERATURE

There are comparatively few references in the literature upon the development of acidity in silage made from crops other than corn.

Esten and Mason (7) have recommended mixing a legume with corn for the purpose of raising the protein content of silage. They asserted that three parts of corn with two parts of cowpeas or soybeans made an excellent combination; also that rye or wheat when mixed with clover made a good silage mixture. They reported that the most progressive farmers of the State were successfully siloing a legume with some member of the grass family. Reports from many States and correspondence with the Experiment Stations indicate that there is a growing tendency to silo cowpeas which have been grown with corn. No data were given to show the type of acid fermentation in any of the above-mentioned crop mixtures.

Recently the Kansas Agricultural Experiment Station (*11, 12*) found that good silage resulted when corn or molasses was mixed with alfalfa in the proportion of 1 to 10 or 1 to 20. The individual acids, however, were not determined, the total acidity being calculated as lactic acid.

[1] Published with the approval of the Director of the Idaho Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 23.

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Upson (*14*), of the Nebraska Agricultural Experiment Station, carried on an investigation with alfalfa and Black Amber sorghum cane mixtures and with alfalfa alone. His results showed excellent silage when alfalfa was mixed with different amounts of Black Amber sorghum cane, but when alfalfa was siloed alone, an undesirable product resulted. His results on the acidity were expressed as total acidity calculated in terms of acetic acid.

True, Woll, and Dolcini (*13*), of the California Experiment Station, have reported favorably upon the practice of cutting the first crop of alfalfa for silage, for the reason that the crop is generally weedy and makes a very inferior hay. Their experience showed that silage made from the first cutting of alfalfa which consisted in a large part of foxtail and other weeds made good silage. The chemical analyses showed that both volatile and nonvolatile acids were present.

A review of the literature indicates that alfalfa can be made into good silage if some material having a high percentage of fermentable carbohydrates is mixed with it.

The siloing of clover has received more favorable comment. Clark (*2*), of the Montana Experiment Station, has summarized the data on clover silage. He reports that at the Agassiz Experiment Station, British Columbia, clover is commonly used for silage. He also states that good results were obtained with it at Pennsylvania State College. The only objection to its use was that a strong odor developed. However, cows ate it readily. Reports from Wisconsin show that tests were made years ago with the uncut plant with unsatisfactory results. Recently more favorable results were obtained when clover was cut in 1-inch lengths and was well tramped in the silo.

EXPERIMENTAL WORK

The work of the Idaho Station on the determination of acids in silage was planned in 1915. At that time only corn silage was available for analysis. The corn was siloed in the fall of 1915 in a large concrete silo of the monolithic type located upon the University farm. The silage had been partially fed to stock before the analysis was begun.

METHOD OF OBTAINING SAMPLES FOR THE ACID DETERMINATION

A composite sample was collected from the various parts of the surface of the silo. One hundred gm. were weighed out and dried to constant weight at 100° C. for the moisture content. The remaining portion of the sample was placed in a hydraulic press and the juice pressed out. To 100 gm. of the juice a small quantity of normal sulphuric acid was added, and the volatile acids separated from the nonvolatile acid by distillation in a current of steam under reduced pressure.

Four liters of distillate were collected and neutralized with *N/10* barium hydroxid and evaporated to a small volume. The volatile acids

were then freed from their barium salts by the addition of a theoretical amount of sulphuric acid. After filtering off the barium-sulphate precipitate, the solution was made up to a definite volume and the volatile acid determined quantitatively. The juice which remained after separating the volatile acids from the nonvolatile acid under reduced pressure was used for the nonvolatile-acid determination.

METHODS USED IN DETERMINING THE ACIDS OF SILAGE

VOLATILE ACIDS

The Duclaux method was used in determining the volatile acids. Until the recently proposed method of Dyer (*5*) it was the only method which proved applicable in the estimation of small amounts of volatile acids when present in a mixture. This method was previously used in studies on corn silage, and since a complete discussion has been taken up under the previous citations, no explanation of the principles involved will be given here. It has been used by numerous investigators in determining volatile acids in known and unknown mixtures. Criticisms have been made of the Duclaux method by Upson, Plum, and Schott (*15*). Some of the criticisms concerning the difficulties involved in carrying the analysis by the Duclaux method the writer fully appreciated in previous work with mixtures of volatile acids. But experiments in determining known mixtures of acids have shown that under the most carefully regulated conditions of distillation, accurate results are obtained and the writer believes that the method deserves greater confidence than given it by the above-mentioned investigators.

Voitkevich (*16*) has obtained results on known mixtures of acetic, propionic, and butyric acids which differed not more than 5 per cent from each other, and this difference is attributed to the variations in the conditions of distillations. He concludes that the method will yield accurate and trustworthy results when carried on under carefully regulated conditions.

Dyer has proposed an excellent method which is more simple to manipulate. The method involves the distillation of the acids in a current of stream from a constant volume. The titration figures are plotted in the form of curves which are characteristic for each acid.

More recently Gillespie and Walters (*9*) published methods for calculating algebraically and graphically the amount of volatile acids in a mixture. The methods of calculation suggested by them are applicable to either the Duclaux or Dyer method, as the methods of calculation are applied irrespective of the mode of distillation. It is merely necessary to conduct all distillations of pure acids and mixtures in the same manner.

COMPARISON OF RESULTS BY THE DUCLAUX AND THE DYER METHOD

A comparison of the Duclaux and the Dyer method was made on a solution of volatile acids obtained from silage in the following manner. Three hundred gm. of expressed juice from silage were acidified slightly with normal sulphuric acid and distilled in a current of steam under reduced pressure. Four liters of the distillate were collected. Five hundred cc. of this distillate were carefully neutralized with *N/10* sodium hydroxid, and evaporated to a small volume. The volatile acids were liberated from their sodium salts by adding the theoretical amount of sulphuric acid. The quantitative determination of volatile acids was then made by Dyer's method.

One thousand cc. of the 4-liter distillate were carefully neutralized with barium hydroxid, evaporated to a small volume, and the acids freed from the barium salts by the addition of the required quantity of normal sulphuric acid. After filtering off the barium sulphate, the solution was made up to volume and the acids determined by the Duclaux method.

A comparison of the results by the two methods is given below.

Volatile acids in 100 gm. of pea-silage juice, as determined by—

	Dyer method.	Duclaux method.
	Gm.	*Gm.*
Acetic acid	0.629	0.601
Propionic acid	.033	.037
Total volatile acids	.662	.638

Volatile acids in corn-silage juice, as determined by—

	Dyer method.	Duclaux method.
	Gm.	*Gm.*
Acetic acid	0.824	0.796
Propionic acid	.072	.082
Total volatile acids	.896	.878

The above results are typical of several determinations of the quantities of volatile acids in the juices of different kinds of silage by the two methods. In all determinations the orientation tests as described by Dyer were made for the individual volatile acids, and their presence or absence was confirmed. The results indicate a slight difference in the proportions of acids found by the two methods; yet this difference easily falls within the limits of experimental error, and it is obvious that either method is applicable for a comparative study of volatile acids in silage.

NONVOLATILE ACID

Lactic acid was determined in the juice that remained after distilling off the volatile acids under reduced pressure. This solution was evaporated on a water bath to a small volume, then extracted with ether in a Bremer continuous extractor for 72 hours. After distilling off the ether, the acid solution was diluted with water, boiled with an excess of barium hydroxid, then exactly neutralized with sulphuric acid. The barium sulphate was filtered off and zinc sulphate added to the filtrate, care being taken to avoid an excess. After filtering off the barium sulphate, the solution was evaporated slowly, the zinc lactate being allowed to crystallize. The crystals were filtered off and washed with a small portion of cold water, then dried, and weighed. A second and sometimes a third crop was obtained from the mother liquor.

OPTICAL ACTIVITY OF LACTIC ACID FROM SILAGE

The combined crops of zinc lactate from each determination were examined for their optical activity. A solution containing at least 4 per cent of zinc lactate in a 2-dm. tube invariably gave a reading of zero degrees. Two gm. of zinc-lactate crystals were also dried in an oven to determine the water of crystallization. Two gm. of zinc lactate gave 0.3635 gm. of water, or 18.17 per cent.

Theoretical water of crystallization for zinc-lactate crystals

	Active form.	Inactive form.
Water of crystallization	12.89	18.18

Although the 2 gm. of zinc lactate were taken from a combined portion of samples obtained from each silo, it is safe to conclude that the zinc lactate was the inactive form. Such results are to be expected in silage fermentation where the conditions of inoculation are not controlled.

EXAMINATION OF SILAGE FROM LARGE SILOS

INVESTIGATIONS OF 1915

CORN SILAGE

Although corn silage has been studied and reported by the Iowa Agricultural Experiment Station (*3, 4, 10*), it was thought best to include corn silage in this investigation. The maturity of the corn is not always the same in this country, owing to early frosts, and since maturity of corn has been stated by investigators to influence the amount of acidity formed, results on acidity of corn produced in Idaho were desired for the sake of comparison. The corn silage was made from a crop of selected Disco Pride corn, cut when the kernels were in the glazed stage. The results are given in Table I.

TABLE I.—*Acidity produced by corn silage in Idaho, 1915*

CONDITIONS OF EXPERIMENT

	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.
Date of sampling	Jan. 26, 1915.	Feb. 11, 1916.	Feb. 17, 1916.	Mar. 2, 1916.	Mar. 7, 1916.
Distance from bottom of silo..feet..	13	10	9	7	6
Moisture..............per cent..	77.7	80.0	77.0	77.8	81.0
Dry material.............do....	23.3	20.0	23.0	22.2	19.0

ACIDS IN 100 GM. OF SILAGE JUICE

	Gm.	Gm.	Gm.	Gm.	Gm.
Acetic acid	0.525	0.766	1.034	1.100	0.946
Propionic acid	.055	.058	.064	.076	.068
Butyric acid	.000	.000	.000	.000	.000
Total volatile acid	.580	.824	1.098	1.176	1.014
Lactic acid	1.141	1.535	1.329	1.595	1.341
Total acidity	1.721	2.359	2.427	2.771	2.355

ACIDS IN 100 GM. OF SILAGE CONTAINING MOISTURE

	Gm.	Gm.	Gm.	Gm.	Gm.
Acetic acid	0.408	0.613	0.796	0.856	0.766
Propionic acid	.043	.046	.049	.059	.055
Butyric acid	.000	.000	.000	.000	.000
Total volatile acid	.451	.659	.845	.915	.821
Lactic acid	.885	1.228	1.024	1.241	1.086
Total acidity	1.336	1.887	1.869	2.156	1.907

ACIDS IN 100 GM. OF SILAGE, DRY BASIS

	Gm.	Gm.	Gm.	Gm.	Gm.
Acetic acid	1.82	3.064	3.462	3.853	4.03
Propionic acid	.19	.232	.214	.266	.290
Butyric acid	.000	.000	.000	.000	.000
Total volatile acid	2.01	3.296	3.676	4.119	4.32
Lactic acid	3.97	6.140	4.460	5.580	5.72
Total acidity	5.98	9.436	8.136	9.699	10.14

INVESTIGATIONS OF 1916

In the work of 1916 the crops used for silage were corn, oats (*Avena sativa*), and peas (*Pisum* spp.), and wheat (*Triticum aestivum*) and peas. These crops were siloed in the three large concrete silos located on the University farm. Particular attention was given to the factors concerned in developing good silage—namely, fineness of cutting, thoroughness of packing, and the proper moisture content.

CORN SILAGE

Corn used for silage in 1916 was the Disco Pride corn, cut when the kernels were in the glazed stage. The amount and kinds of acid are given in Table II.

OATS AND PEAS

The oat and pea silage was made from a crop of white Canada field peas and Swedish Select oats sown at the rate of 60 pounds of the former and 40 pounds of the latter per acre. The crop was cut when the peas were beginning to harden in the pods and when the oats were in the dough stage. The results on acidity are given in Table II.

WHEAT AND PEAS

The wheat and pea mixture was made from a crop of white Canada field peas and Palouse Bluestem wheat sown at the rate of 75 pounds of the former and 25 pounds of the latter per acre. The peas grew luxuriantly, and the green weight of the peas exceeded considerably the green weight of the wheat. The crop was cut when the peas were beginning to harden in the pods and when the wheat was in the dough stage. The data on acidity are given in Table II.

TABLE II.—*Acidity produced by corn silage, oat and pea silage, and wheat and pea silage in large silos, Idaho, 1916*

Acid.	CORN SILAGE. (Date of sampling, Dec. 4, 1916; moisture, 77 per cent; dry material, 23 per cent; location, 15 feet from top of silo).			OAT AND PEA SILAGE. (Date of sampling, Oct. 6, 1916; moisture, 76 per cent; dry material, 24 per cent).			WHEAT AND PEA SILAGE. (Date of sampling, Nov. 11, 1916; moisture, 73.3 per cent; dry material, 26.7 per cent; location, 6 feet from bottom of silo).		
	Acidity in 100 gm. of—			Acidity in 100 gm. of—			Acidity in 100 gm. of—		
	Silage juice.	Silage containing original moisture.	Silage on dry basis.	Silage juice	Silage containing original moisture.	Silage on dry basis.	Silage juice.	Silage containing original moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	1.022	0.787	3.46	0.542	0.396	1.47	0.718	0.526	1.97
Propionic	.053	.041	.18	.038	.028	.10	.052	.038	.14
Total volatile.	1.075	.828	3.64	.580	.424	1.57	.770	.564	2.11
Lactic	1,280	.986	4.33	1.690	1.234	4.57	1.420	1.048	3.90
Total acidity.	2.355	1.814	7.97	2.270	1.660	6.14	2.190	1.612	6.01

DISCUSSION OF RESULTS

CORN SILAGE.—All samples of corn silage examined in 1915 and 1916, showed the usual acid fermentation. There was more lactic acid formed than acetic and propionic acids, and butyric acid was absent from all

samples. Considerable more acidity was developed in this corn silage made in Idaho than in that studied previously at the Iowa Station. But this is partially accounted for by the fact that corn does not usually reach as high a state of maturity in this section. These results corroborate previous statements by certain investigators that immature corn will produce a silage higher in acidity than mature corn.

OAT AND PEA SILAGE.—Oat and peas made first-class silage. It had a good color and odor and showed an acid fermentation similar to corn silage.

WHEAT AND PEA SILAGE.—Wheat and peas made very good silage showing all the characteristics of a normal silage. The kinds and quantity of acids developed were similar to those found in corn silage.

EXAMINATION OF SILAGE FROM SMALL SILOS

In 1916 additional work was done with crop mixtures other than corn siloed in small wooden stave silos of approximately 1,500 pounds' capacity. In these silos peas and oats were siloed alone and in definite proportions, based on the dry weight of each, as were also clover and alfalfa, and definite mixtures of each, with wheat straw. Oats and peas were chosen because they were likely to be more commonly used than any other crops or crop mixture as corn substitutes. Clover and alfalfa were chosen because there are some sections where the first cuttings are cured with difficulty for hay because of rainy weather, and there has been some inquiry as to the possibility of converting them alone and in mixtures into silage. It seemed worth while to ascertain whether the same type of fermentation could be depended upon from mixtures of these legumes with crop residues that are all too frequently allowed to go completely to waste. The small silo series was filled with crops and mixtures as indicated below:

Peas 100 per cent.
Oats 100 per cent.
Clover 100 per cent.
Alfalfa 100 per cent.
Peas 87½ and oats 12½ per cent.
Peas 75 and oats 25 per cent.
Peas 50 and oats 50 per cent.
Clover 75 and wheat straw 25 per cent.
Clover 50 and wheat straw 50 per cent.
Alfalfa 75 and wheat straw 25 per cent.
Alfalfa 50 and wheat straw 50 per cent.

These minature silos were made of 2-inch fir staves. They were 3 feet in diameter and 6 feet in height. The staves were drawn closely together by means of lugs attached to iron bands. To make them perfectly air- and water-tight, the joints were coated on the inside with hot paraffin.

Before filling the silos, moisture determinations were made on the green materials in order to weigh out the proper amount. The cut materials were mixed uniformly on the floor and then packed in small

silos. One man was kept busy tramping and water was added when necessary in sufficient quantities to raise the average moisture content to 75 per cent. It was not necessary to add water to the peas, clover, and alfalfa when siloed alone.

When the silo was filled, a tightly fitting lid made of 2-inch fir plank was placed on the silage and 800 pounds of brick were evenly distributed on the lid. This pressure insured the proper settling of the silage, and made the conditions very similar to those found in a big silo, and in addition reduced the spoiled silage to a minimum.

Babcock and Russel (*1*) assert that silage made in small containers will be equal to the silage made in large silos, if conditions of siloing are properly controlled. Eckles and his collaborators (*6*) in their investigation on corn silage used small wooden silos 3 feet in diameter and 6 feet in height with the addition of a weight on the top of the silo to bring the silage under similar conditions as are found in the large silos. Their conclusions were as follows:

> A comparison of silage from a large silo and of silage from the same corn put into a small experimental silo showed the quality to be the same, as judged by appearance and by chemical analysis. For all purposes, except studying temperature changes, the small silo is believed sufficiently accurate for experimental purposes.

State of Maturity of the Different Crops

Peas were cut at the time the peas were beginning to harden in the pod, and the foliage around the bottom just beginning to turn brown.

Oats were cut when the kernels were in the dough stage.

Clover was the first cutting, cut at the stage when a few of the blossoms were beginning to turn brown.

Alfalfa was also from the first cutting, cut at the time when the new shoots began to appear.

Wheat straw used in the clover and alfalfa series contained a small quantity of wheat.

Examination of Silage

All silos were allowed to remain closed for a period of three months, with the exception of the clover silo, which was closed for four months. In all cases sufficient time elapsed to insure a complete acid fermentation. Samples were obtained by boring into the silos at a height of 3 feet from the floor, and by means of the auger removing quantities sufficient for the analyses. The holes in the sides of the silos were then stoppered with wooden plugs.

The silage samples were treated in the same manner as described in the early part of this paper for the determination of volatile and nonvolatile acids.

TABLE III.—*Acidity produced by pea silage, oat silage, oat and pea silage, clover silage, clover and wheat-straw silage, alfalfa silage, alfalfa and wheat-straw silage in small silos, Idaho, 1916*

Acid.	PEA SILAGE. (Moisture, 76 per cent; dry material, 24 per cent.)			OAT SILAGE. (Moisture, 70.4 per cent; dry material, 29.6 per cent.)			OAT AND PEA SILAGE (PEAS, 87½ PER CENT; OATS, 12½ PER CENT, DRY BASIS). (Moisture, 76.2 per cent; dry material, 23.8 per cent.)		
	Acidity in 100 gm. of—			Acidity in 100 gm. of—			Acidity in 100 gm. of—		
	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	0.599	0.455	1.897	0.378	0.266	0.881	0.886	0.675	2.836
Propionic	.043	.033	.137	.020	.014	.046	.064	.049	.205
Total volatile	.642	.488	2.034	.398	.280	.927	.950	.724	3.041
Lactic	1.902	1.446	6.02	1.746	1.229	4.07	1.474	1.123	4.72
Total acidity	2.544	1.934	8.054	2.144	1.509	4.997	2.424	1.847	7.761

Acid.	OAT AND PEA SILAGE (PEAS, 75 PER CENT; OATS, 25 PER CENT, ON DRY BASIS). (Moisture, 74 per cent; dry material, 26 per cent.)			OAT AND PEA SILAGE (PEAS, 50 PER CENT; OATS, 50 PER CENT, DRY BASIS). (Moisture, 74.3 per cent; dry material 25.7 per cent.)			CLOVER SILAGE. (Moisture, 75 per cent; dry material, 25 per cent.)		
	Acidity in 100 gm. of—			Acidity in 100 gm. of—			Acidity in 100 gm. of—		
	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	0.760	0.562	2.148	0.681	0.506	1.969	0.9	0.675	2.70
Propionic	.034	.040	.154	.064	.048	.184	.056	.042	.168
Total volatile	.814	.602	2.302	.745	.554	2.153	.956	.717	2.868
Lactic	1.772	1.311	5.01	1.592	1.183	4.602	.860	.645	2.580
Total acidity	2.586	1.913	7.312	2.337	1.737	6.755	1.816	1.362	5.448

Acid.	CLOVER AND WHEAT-STRAW SILAGE (CLOVER, 75 PER CENT; STRAW, 25 PER CENT). (Moisture, 73.5 per cent; dry material, 26.5 per cent.)			CLOVER AND WHEAT-STRAW SILAGE (CLOVER, 50 PER CENT; STRAW, 50 PER CENT). (Moisture, 75 per cent; dry material, 25 per cent.)			ALFALFA SILAGE. (Sample taken 3 months after siloing; moisture, 75 per cent; dry, material 25 per cent.)		
	Acidity in 100 gm. of—			Acidity in 100 gm. of—			Acidity in 100 gm. of—		
	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	0.884	0.650	2.450	0.674	0.506	2.028	1.253	0.940	3.76
Propionic	.064	.047	.179	.063	.047	.188	.153	.115	.46
Total volatile	.948	.697	2.629	.737	.553	2.215	1.406	1.055	4.22
Lactic	.966	.710	2.870	.927	.695	2.781	Trace.	Trace.	Trace.
Total acidity	1.914	1.407	5.499	1.664	1.248	4.997	1.406	1.055	4.22

TABLE III.—*Acidity produced by pea silage, oat silage, oat and pea silage, clover silage, clover and wheat-straw silage, alfalfa silage, alfalfa and wheat-straw silage in small silos, Idaho, 1916*—Continued

Acid.	ALFALFA AND WHEAT-STRAW SILAGE (ALFALFA, 75 PER CENT; STRAW, 25 PER CENT). (Moisture, 75 per cent; dry material, 25 per cent.) Acidity in 100 gm. of—			ALFALFA AND WHEAT-STRAW SILAGE (ALFALFA, 50 PER CENT; STRAW, 50 PER CENT). (Moisture, 75.5 per cent; dry material, 24.5 per cent.) Acidity in 100 gm. of—		
	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	0.458	0.344	1.375	0.646	0.488	1.992
Propionic	.328	.246	.983	.058	.044	.180
Butyric	.724	.543	2.172	.389	.294	1.198
Total volatile	1.510	1.133	4.530	1.093	.826	3.370
Lactic	.000	.000	.000	.000	.000	.000
Total acidity	1.510	1.133	4.530	1.093	.826	3.370

PEA SILAGE

When opened, the pea silage had a very pleasant odor, but was a little dark in color. The determination of acids showed that a fermentation had taken place which was similar to that found in normal corn silage (Table III).

OAT SILAGE

The oat silage had a very fine color and odor. Dairy stock ate it with relish. The acid fermentation was normal and similar to that in corn silage (Table III).

OAT AND PEA SILAGE

The three combinations of oats and peas made excellent silage from the standpoint of acid fermentation. In every instance, the silage had a good color and odor, and the stock ate it readily. In so far, then, as the acid fermentation is concerned, the proportion of the two crops used makes practically no difference. This fact is of considerable importance to the man who might wish to grow the greatest tonnage possible per acre by comparatively heavy sowings of peas (Table III).

CLOVER SILAGE

The clover silage was sampled four months after filling the silo. The silage was quite dark in color, but had an agreeable odor. The acid fermentation was similar in the kind of acids developed, but the proportion of volatile ands nonvolatile acid varied from that found in normal corn silage. The dairy stock ate this silage with relish (Table III).

CLOVER AND WHEAT-STRAW SILAGE

The appearance of clover and clover and wheat straw mixture was very similar. Both kinds of silage kept well, and practically no difference in color or odor could be noted. It appeared that dairy stock ate the clover and wheat-straw silage with more relish, but both kinds were entirely consumed at each feeding. The acid fermentation in both cases was similar to that in corn silage. Siloing a portion of wheat straw with clover offers an opportunity for securing the maximum food value from wheat straw that otherwise is generally burned in the stack (Table III).

ALFALFA SILAGE

In a sample of silage made from alfalfa alone taken three months after siloing no butyric acid was found. Moreover, lactic acid was absent. A sample taken nine months after siloing had a strong odor of butyric acid. It was examined qualitatively by the orientation tests suggested by Dyer, and the presence of butyric acid was confirmed. The silage was unfit for feeding purposes. This fact showed that alfalfa silage gradually deteriorates with age and confirms the conclusions of Reed and Fitch (*11*), who state that alfalfa can be made into silage if it is fed soon after siloing, but on standing it rapidly becomes unfit for feeding purposes. Experiments are now under way to determine the practicability of adding crude glucose to alfalfa when siloed for the purpose of raising the percentage of fermentable carbohydrates, the object being to insure an acid fermentation that is similar to that in corn silage.

Reed and Fitch state that—

> There is as much acid produced in alfalfa silage as in kafir or cane silage. This would indicate that the acid content of silage is not always an index to the quality of silage.

In the author's opinion the criterion of good silage is the kind of acids present rather than the quantity. Good silage must have a sufficient quantity to insure its keeping, but beyond this point silage may vary in amount of acidity and yet be classed as normal silage. All good silage examined by the writer contained lactic, acetic, and propionic acids, and in almost all cases lactic acid was in excess of the sum of acetic and propionic acids. It is assumed that no determinations were made by Reed and Fitch on the kinds of acids present in alfalfa silage, but that all the acidity was calculated in terms of lactic acid. Obviously, by this method it would appear to them that quantity of acids was not a determining factor.

The author found that in alfalfa and other silage of poor quality butyric acid was always present in amounts varying with the degree of spoiling that it had undergone. Moreover, in all samples of alfalfa silage lactic acid was found only in traces. It is hard to explain why lactic acid is absent in alfalfa silage, when it is usually the predominating

acid in good silage. There is a possibility that alfalfa, lacking sufficient carbohydrate, first develops lactic acid, which is in turn reduced to propionic acid and by further reductions to butyric acid by microorganisms. Such reactions have been shown by Fitz (*8*) to be possible by certain organisms. However, no study has been made of the acids of alfalfa silage developed during the first few days after siloing, which is necessary to determine this point. The suggestion, therefore, is only tentative. It is also possible that some butyric acid develops at the expense of the proteins. Therefore poor silage may actually contain as much acid as a silage of good quality, but may, nevertheless, be unfit for feeding purposes.

ALFALFA AND WHEAT-STRAW SILAGE

The alfalfa and wheat straw combinations did not make good silage. The silage had a very disagreeable odor and was not fit for feeding purposes. A glance at Table III shows that the acid fermentation differed from the fermentation that takes place in good silage.

TABLE IV.—*Summary of volatile and nonvolatile acids in silage made from forage crops in small silos*

[Results are given on 100 gm. of silage, dry basis]

Silage.	Acetic acid.	Propionic acid.	Butyric and volatile acids.	Total volatile acids.	Lactic acid.	Total acidity.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Alfalfa	3. 76	0. 469	....	4. 229	0. 000	4. 229
Alfalfa ....75 per cent. / Wheat straw...25 per cent.	1. 375	. 983	2. 172	4. 53	. 000	4. 53
Alfalfa ....50 per cent. / Wheat straw...50 per cent.	1. 992	. 180	1. 198	3. 37	. 000	3. 37
Clover	2. 70	. 168	....	2. 868	2. 580	5. 448
Clover ....75 per cent. / Wheat straw...25 per cent.	2. 45	. 179	....	2. 629	2. 870	5. 499
Clover ....50 per cent. / Wheat straw...50 per cent.	2. 028	. 188	....	2. 216	2. 781	4. 997
Peas	1. 897	. 137	....	2. 034	6. 02	8. 054
Peas ....87½ per cent. / Oats ....12½ per cent.	2. 836	. 205	....	3. 041	4. 72	7. 761
Peas ....75 per cent. / Oats ....25 per cent.	2. 148	. 154	....	2. 302	5. 01	7. 312
Peas ....50 per cent. / Oats ....50 per cent.	1. 969	. 184	....	2. 153	4. 602	6. 755
Oats	. 881	. 046	....	. 927	4. 07	4. 997

SUMMARY

(1) Previous investigations showed that all samples of high-class corn silage contain lactic, acetic, and propionic acids, the nonvolatile lactic acid usually occurring in excess of the sum of the volatile, acetic, and propionic acids. Of the volatile acids, acetic is greatly in excess of the propionic acid.

(2) The crops and crop mixtures under examination which showed an acid fermentation similar to corn silage and were all first-class silage are as follows:

Oats and peas in any proportion.	Wheat and peas.
Oats.	Clover.
Peas.	Clover and wheat straw.

(3) Crops and crop mixtures under examination which did not develop an acid fermentation similar to corn, and were unfit for feeding purposes are as follows:

Alfalfa, unless fed soon after siloing.
Alfalfa and wheat straw.

(4) Butyric acid was always found in samples of spoiled or partly spoiled silage.

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PIÑON BLISTER-RUST

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INTRODUCTION

A species of Cronartium on *Ribes aureum* has been known in Colorado for many years, having been collected by one of the writers (Bethel) in the Ute Indian Reservation in southwestern Colorado in 1897, and in 1909 at Boulder and Denver, and various other places since that time.[2]

Since this species of Cronartium closely resembles *Cronartium ribicola* Fischer de Wald, the cause of the white-pine blister-rust, and likewise attacks species of Ribes, and because no species of caulicolous Peridermium has hitherto been known in Colorado which could serve as its æcial stage, it has been considered by some to be identical with *C. ribicola*. On the other hand, the occurrence of this rust at Boulder, Colo., on *Ribes aureum* for several years in close proximity to several white pines (*Pinus strobus*) which remained uninfected indicated that it was not *C. ribicola*.

The earliest collection of this western species of Cronartium is now believed to be one of the uredinial stage collected by Bartholomew and reported by Arthur[3] (*2, p. 130*) in 1892 in Kansas. This was at first identified by Arthur (*7, p. 63*) as presumably *Cronartium ribicola* Arthur, upon receiving data and specimens of Mr. Bethel's collections in 1909, wrote him as follows:[4]

> The Cronartium on Ribes from Boulder is an interesting species. I think you are right in regarding it as an undescribed form, and this accounts for the uredo on Ribes found in Kansas, which is reported under *Cronartium ribicola* in the North American Flora (*1*).

Spaulding (*6*) in 1911 referred to the Kansas collection as *Cronartium ribicola*, basing his action on Arthur's first determination (7). Later, on seeing a specimen of the collection in Kansas by Bartholomew, Spaulding considered it a distinct species. Arthur and Kern (*2*), in 1914,

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[1] The writers are under obligations to Mr. F. V. Coville, of the office of Economic and Systematic Botany, Bureau of Plant Industry, for the determination of species of Ribes; to the State Historical and Natural History Society, Denver, Colo., for office room and other courtesies; to the officers of District 2, Forest Service, Denver, for cooperation in securing information regarding forest conditions; and to Mr. Hugh McCeary and Mrs. S. N. Keith, Bayfield, Colo., and Mr. R. Branson, Mancos, Colo., for material assistance in expediting the work of investigation.

[2] As shown by correspondence in the files of this Office, Mr. Bethel made collections at other places in Colorado previous to 1909, but the material is stored and not available for examination. No localities are listed in this paper unless the specimens collected have been recently examined by the writers.

[3] Reference is made by number (italic) to "Literature cited," pp. 423-424.

[4] Letter to Mr. Bethel dated Nov. 11, 1909.

treated this species under *C. ribicola*, but stated that its occurrence on *Ribes* (*longiflorum*) *aureum* in the parks of Denver and Boulder was unexplainable. That this rust is not *C. ribicola* was predicted by Hedgcock and Long (4) in 1915 and by Bethel in 1917 in North American Uredinales No. 1609.

In connection with the white-pine blister-rust investigations [1] during the summer of 1917, a thorough investigation of the rust was begun. The species of caulicolous Peridermiums previously known in Colorado and Arizona are *Peridermium pyriforme* Peck, *P. filamentosum* Peck, and *P. harknessii* Moore, all of which attack *Pinus ponderosa* and *P. contorta*, and whose telial stages are well known. The first problem, therefore, was to find a species of Peridermium which could be shown to be the æcial stage of this species of Cronartium. Since the northern and central parts of the State had long been a favorite collecting ground of mycologists and no such species of Peridermium had been discovered, it seemed probable that it would be found in the southern part of the State, where less collecting had been done. The first collection of this species of Cronartium had been made in the Ute Indian Reservation along Los Pinos River near Bayfield, about 20 miles north of the Colorado-New Mexico boundary; so this region was surveyed first.

On May 25, 1917, the senior writer found a Peridermium, apparently a new species, on *Pinus edulis* south of Bayfield. The following day two of the writers (Hedgcock and Bethel) found the telia of the Cronartium on fallen leaves of *Ribes aureum* north of Bayfield, near the spot where Mr. Bethel had collected the fungus in 1897. Culture material of the species of Peridermium was sent to Washington, D. C., where another of the writers (Hunt) made inoculations with æciospores on species of Castilleja, Comandra, and Ribes, with the result that *R. odoratum* became infected with the species of Cronartium, establishing the new species of Peridermium as the æcial stage of the western species of Cronartium. Repeated inoculations given elsewhere in this paper have fully corroborated this result.

This species of Cronartium was first found in Arizona by Mr. Goodding on October 2 at Prescott, Ariz., and was at first supposed to be *C. ribicola*. Dr. Long, assisted by Messrs. Goodding and Llewellyn, made a thorough survey of the region around Prescott in October and found this species of Cronartium abundant on both cultivated and wild plants of *Ribes* spp. in several localities, some widely separated, but did not find the æcial form on pines. On October 28 one of the writers (Bethel) found one tree of piñon (*Pinus monophylla*) near Prescott dis-

[1] The work in Colorado was in charge of Dr. G. G. Hedgcock, assisted by Messrs. E. Bethel, N. R. Hunt, E. B. Payson, E. L. Johnson, R. Thompson, and H. L. Gaymon. Dr. W. H. Long was in charge in New Mexico and Arizona, assisted by Messrs. C. S. Lewellyn and L. N. Goodding. Mr. A. S. Rhoads assisted in the work of inoculation at Washington, D. C. The work in Colorado was in cooperation with the Colorado Agricultural Experiment Station, which was in charge of the inspection of nursery stock and nurseries for the white-pine blister-rust.

eased with the æcial form, then past maturity. Old æciospores were found to be present by Messrs. Hedgcock and Bethel. This tree was examined by Messrs. Bethel and Hunt in May, 1918, but new, fresh æcia had not been formed. Old æciospores were still present. Messrs. Hedgcock and Bethel collected specimens of the *Cronartium* sp. at several points around Prescott during the latter part of October, 1917, and pronounced it distinct from *C. ribicola*, but identical with the species of Cronartium previously found at Bayfield and other localities in Colorado.

DESCRIPTION OF THE FUNGUS

The morphology of this new species of Peridermium differs so much from that of *P. strobi* Kleb., the æcial stage of *Cronartium ribicola*, that the writers are led to believe it is the æcial stage of a distinct species. The Colorado species of Cronartium on *Ribes* spp. is here designated "*Cronartium occidentale*," with the following description:

Cronartium occidentale, n. sp. (Pl. 54, 56, 57).

O.—Pycnia caulicolous, scattered, forming blisters 0.5 cm. or more in diameter; exudate orange-chrome;[1] pycniospores hyalin, pyriform or obovoid to ellipsoid, 2 to 3 by 3 to 5 μ, averaging 2 by 4 μ; pycnial scars seldom found (Pl. 56).

I.—Æcia caulicolous, sometimes causing slight hypertrophy; æcial cavities large, often entirely hidden by the bark; peridia inconspicuous, thin, evanescent, only slightly protruding, if at all, rupturing at irregular cracks in the bark or near the top if protruding; peridial cells variable, nearly smooth on outer surface, verrucose on inner surface, 12 to 26 by 17 to 36 μ, averaging 19 by 25 μ, walls 1 to 5 μ thick; æciospores variable, usually obovoid to ellipsoid, 12 to 28 by 22 to 38 μ, walls colorless, 1 to 5 μ thick, averaging 3 μ, outer wall coarsely verrucose, with deciduous tubercules, which in end view are 0.7 to 2.5 by 1 to 4.6 μ, averaging 1.4 by 1.8 μ, in side view 1.7 to 4.2 μ, averaging 2.7 μ long (Pl. 54).

In Arizona on *Pinus monophylla*.

In Colorado on *Pinus edulis*.

The type specimen of the æcial stage is FP 26227 [2] on *Pinus edulis*, collected by Messrs. E. Bethel and H. L. Gaymon at Bayfield, Colo., on July 13, 1917.

II.—Uredinia hypophyllous, rarely amphigenous, scattered on irregularly-rounded areas; sori light yellow to yellow, pustular, 0.2 to 2 mm. in diameter, dehiscent by a central opening; urediniospores 13.5 to 20 by 18.5 to 32 μ, averaging 16 by 24 μ, with colorless walls 2 to 3 μ thick, sharply echinulate on outer surface.

III.—Telial columns hypophyllous or rarely amphigenous, and occasionally on the petioles and peduncles, cylindrical or nearly so, 60 to 170 μ thick, up to 4 mm. long, walnut-brown to Vandyke-brown; teliospores oblong to cylindric, 9 to 19 by 27 to 56 μ; wall nearly colorless, 0.4 to 2 μ thick (Pl. 57).

In Arizona on *Ribes aureum*, *R. odoratum*, and *Grossularia reclinata*×*G. hirtella*.[3]

In Colorado on *Ribes aureum*, *R. inebrians*, and *Grossularia leptantha*.

In Washington, D. C., by cultures on *Ribes americanum*, *R. aureum*, *R. coloradense*, *R. giraldi*, *R. glandulosum*, *R. malvaceum*, *R. nigrum*, *R. odoratum*, *R. sanguineum*, *R. sp.*, *Grossularia inermis*, *G. missouriensis*, and *G. reclinata*×*G. hirtella*.

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[1] Color terms used are from RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 pl. (col.). Washington, 1912.

[2] The type specimens of *Cronartium occidentale* are deposited in the Pathological Collections of the United States Department of Agriculture at Washington, D. C.

[3] The type specimen of the uredinial and telial stages is FP 24420 on *Ribes aureum* collected by Messrs. Bethel and Hunt at Bayfield, Colo., on September 15, 1917.

For the convenience of those who wish to compare *Cronartium occidentale* with *C. ribicola* the principal points of difference are shown below in parallel columns. The characteristics of *C. ribicola* are furnished by Dr. Perley Spaulding and Dr. R. H. Colley, of this Office.

Cronartium occidentale differs from *C. ribicola* in its pycnial and æcial stages, in its æcial hosts, and in its behavior on some telial hosts as follows:

CRONARTIUM OCCIDENTALE	CRONARTIUM RIBICOLA
O.—PYCNIAL EXUDATE orange chrome; PYCNIAL SPOTS uncommon and not conspicuous.	O.—PYCNIAL EXUDATE honey-yellow; PYCNIAL SPOTS numerous and conspicuous.
I.—ÆCIAL AREAS showing slight, if any hypertrophy (Pl. 54). ÆCIOSPORES borne in large cavities, peridia seldom prominent, often not visible (Pl. 54). PERIDIUM, thin, evanescent; SPORES usually released through irregular cracks in the overlying bark; SPORES 12 to 28 by 22 to 38 μ; wall 1 to 5 μ, averaging 3.0 μ thick.	I.—ÆCIAL AREAS usually showing a marked fusiform swelling, especially on younger trees (Pl. 55). ÆCIOSPORES borne within closely aggrerated, prominently protruding peridia (Pl. 55). PERIDIUM, thick, persistent; SPORES released by irregular breaking of the peridia. SPORES 18 to 20 by 22 to 33 μ, wall 2.0 to 2.5 μ thick.
O and I.—On piñon (1- and 2-needled) pines.	O and I.—On white (5-needled) pines.
I and II.—Incubation period on *Ribes* spp. 12 to 36 days.	I and II.—Incubation period on *Ribes* spp. 5 to 14 days.
II and III.—*Ribes nigrum*, one of the poorest hosts in inoculations. *Grossularia leptantha* best host in at least one locality in Colorado.	II and III.—*Ribes nigrum* best host. *Grossularia leptantha* poorest host in inoculations.
Native of America.	Native of Old World.

The striking differences in the appearance and morphology of the æcial stages of *Peridermium occidentale* and *Peridermium strobi* might be thought to be due, in part at least, to differences in the bark of the hosts, since *Pinus edulis* has a rather thick bark while *Pinus strobus* and other white pines have a thin bark. However, *Peridermium harknessii*, which sloughs off the overlying bark tissues in much the same way as *Peridermium occidentale*, has the same characteristics whether found on the thin-barked *Pinus contorta* or the thick-barked *Pinus ponderosa*. *Peridermium pyriforme*, which produces numerous protruding peridia much like those of *Peridermium strobi*, also occurs on *Pinus contorta* and on *Pinus ponderosa*, with no apparent modification resulting from the variation in the thickness of the bark. *Peridermium filamentosum* on the same hosts behaves similarly.

Although it was soon evident that the incubation period on *Ribes* spp. was longer for *Cronartium occidentale* than for *C. ribicola*, only one test has been made for the specific purpose of comparison on this point. On November 14 four very similar plants of *Grossularia inermis*

and two of *Ribes odoratum* were selected from the stock beds. One half of these plants were inoculated with *C. occidentale* by Hunt and the others with *C. ribicola* by Mr. G. F. Gravatt, of this Office. All plants were covered with bell jars and put under a side bench of the greenhouse. A tight glass and wood partition separated the two lots of plants, but the most widely separated plants were less than 6 feet apart.

On November 21 the plants of *Grossularia inermis* inoculated with *Cronartium ribicola* were producing urediniospores. On November 22, urediniospores were abundant on these plants, and some were being produced by part of the numerous uredinia on the plant of *Ribes odoratum*. A careful examination with a hand lens failed to reveal any uredinia in process of formation on the plants inoculated with *C. occidentale*, although these plants later developed a heavy infection, first producing a few spores on November 27. Abundant uredinia and telia were produced on all of the plants inoculated. The difference in the length of the incubation period is variable but marked, and this fact supports the view that the two forms are distinct species.

To summarize briefly, *Cronartium occidentale* differs essentially from *C. ribicola* as follows: In the Peridermium or æcial stage the former bears its thicker-walled æciospores in a few large cavities under the bark and bordered with thin and evanescent peridia, while those of the latter are borne in numerous small cavities beneath thicker-walled, protruding, persistent peridia; the pycnial stage of the former has an orange-chrome pycnial exudate, the latter a honey-yellow one; the incubation period from the æcial stage to the uredinial stage is longer in the former than in the latter; the telial stage of the former infects *Grossularia leptantha* abundantly and *Ribes nigrum* rarely and sparsely, while the reverse is true in the case of the latter.

DISTRIBUTION OF CRONARTIUM OCCIDENTALE

DISTRIBUTION OF THE ÆCIAL STAGE

The æcial stage is here designated "*Peridermium occidentale*" to distinguish it from the æcial stages of other species of the form-genus Peridermium. It has been found only in two States, Arizona and Colorado (fig. 1), as follows:

ARIZONA:

On *Pinus monophylla*.—Near **Prescott**,[1] HEDGCOCK and BETHEL, October 28.[2]

COLORADO:

On *Pinus edulis*.—**Bayfield**, HEDGCOCK and BETHEL, May 25 (first collection of æcial stage), BETHEL and GAYMON, June 23, 28, and 30, July 12 and 13, BETHEL, August 26 and 31; **Cedar Creek**, Montrose County, BETHEL, July 31; **Glenwood Springs**, PAYSON, August 31, and HUNT and BETHEL, October 3; **Mancos**, BETHEL and PAYSON, August 16, BETHEL, August 17, and BETHEL and HUNT, September 19; **Mesa Verde National Park**, BETHEL and HUNT, September 21.

[1] All data on distribution are supported by numbered specimens in our collection.

[2] For data with the year omitted refer to the year 1917.

DISTRIBUTION OF THE UREDINIAL AND TELIAL STAGES

The uredinial and telial stages have been collected as follows:

ARIZONA:[1]

On *Ribes aureum*.—**Copper Basin,** LONG, October 6; **Cottonwood,** HEDGCOCK and BETHEL, October 31; **Prescott,** GOODDING, September 27, LONG and GOODDING, October 6, LONG, October 6, HEDGCOCK, LONG, BETHEL, and LLEWELLYN, October 26 and 27, and HEDGCOCK and BETHEL, October 29; **Walnut Creek,** LLEWELLYN, October; **Verde River Valley,** LLEWELLYN, October.

On *Ribes odoratum*.—**Prescott,** GOODDING, October 2, LONG and GOODDING, October 6, LONG, October 6 and 11, and HEDGCOCK and BETHEL, October 29.

On *Grossularia reclinata* × *G. hirtella*.—**Prescott,** LONG and GOODDING, October 6, LONG, October 10, and HEDGCOCK, LONG, BETHEL, and LLEWELLYN, October 26 and 27.

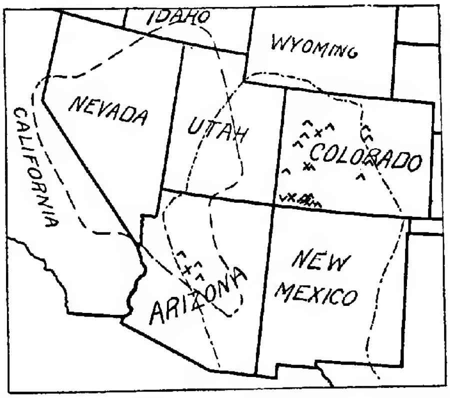

FIG. 1.—Outline sketch map showing the distribution of *Pinus edulis* and of *P. monophylla* in the United States. The former is included in the broken line of dashes and dots to the right, and the latter by the line of dashes to the left. In this region is the known distribution of *Cronartium occidentale*. Localities where collections of the different stages of the fungus have been made are indicated on the map as follows: V, æcial stage on species of pine; Λ, uredinial and telial stages on species of Ribes: ×, all forms present.

COLORADO:

On *Ribes aureum*.—**Boulder,** BETHEL, August, 1909, August 1, 1911, September, 1913, July 1, August 1, and August 4, 1914, HEDGCOCK, October 7 and 17, 1914, October 13, 1916; **Bayfield,** BETHEL, August 3, 26, and September 12; **Canon City,** HUNT and BETHEL, October 5; **Cedar Creek,** W. W. ROBBINS, October, 1912, and BETHEL, July 9; **Colorado Springs,** HUNT and BETHEL, October 13; **Cimarron,** HEDGCOCK, October 10 and 11; **Denver,** BETHEL and HUNT, October 10 and 16, HEDGCOCK and BETHEL, October 17, HEDGCOCK and THOMSON, August 11, HEDGCOCK, October 17, 1914, October 11, 1916, September 25; **Devils Creek,** BETHEL and PAYSON, August 11, BETHEL, August 26 and 27, BETHEL and HUNT, September 8 and 24; **Debeque,** HEDGCOCK, October 16; **Durango,** BETHEL and HUNT, September 17; **Florida River,** BETHEL and HUNT, September 15; **Glenwood Springs,** HEDGCOCK, BETHEL and HUNT, October 4; **Hermosa,** BETHEL and HUNT, September 16; **Los Pinos River,** BETHEL, July, 1897 (first collection of telial stage); **Mancos,** BETHEL and PAYSON, August 16, and BETHEL and HUNT, September 22; **McCoy,** HEDGCOCK, September 28; **Meeker,** HEDGCOCK, October 2; **Naturita,** PAYSON, July 24 and August 18; **Piedra,** BETHEL and PAYSON, August 11; **Rifle,** HEDGCOCK, October 3, 5, and 6.

On *Ribes inebrians*.—**Glenwood Springs,** PAYSON. August 31.

On *Grossularia leptantha*.—**Cimarron,** HEDGCOCK, October 11; **Glenwood Springs,** PAYSON, August 29, BETHEL and HUNT, October 3, and HEDGCOCK, October 4.

[1] All Arizona collections were made in Yavapai County.

DISTRICT OF COLUMBIA, WASHINGTON;[1]

On *Ribes americanum*.—HUNT, November 11:

On *Bibes aureum*.—HEDGCOCK, August 31, September 8, 19, and 21, and HUNT, November 3.

On *Ribes giraldi*.—HEDGCOCK, September 21, and HUNT, November 3.

On *Ribes malvaceum*.—HEDGCOCK, September 11, and HUNT, November 3.

On *Ribes nigrum*.—HUNT, November 3.

On *Ribes* sp.—(near *R. aureum*) HEDGCOCK, September 21.

KANSAS:

On *Ribes aureum*.—Rooks County, E. BARTHOLOMEW, August 22, 1892. (First collection of uredinial stage.)

Although the survey for *Peridermium occidentale* was continued in Colorado throughout the summer of 1917 until late in October, only 42 trees of *Pinus edulis* were found diseased by it. These were found in five widely separated localities. In Arizona only one diseased tree of *Pinus monophylla* was found late in the season. The finding of telia on *Ribes aureum* in Arizona in the vicinity of trees of *Pinus cembroides* indicates that this also may be an æcial host. No survey was made of regions where *Pinus quadrifolia* is found. These four species of pine are known as piñon pines. Since this rust is the only one known to occur on the stems of piñons, and is, so far as known, confined to piñons, it is called the "piñon blister-rust."

Peridermium occidentale has not been found on the native white pines *Pinus aristata* and *P. flexilis*, although both species occur frequently in association with *Grossularia leptantha* and *Ribes inebrians*, which are native hosts for the Cronartium. These species of Grossulariaceae often range from the piñon belt, where *P. edulis* abounds, to the higher altitudes, where the white pines occur, furnishing a means for spreading the rust in the uredinial and telial stage from the former species of pine to the latter if they were susceptible.

Cronartium occidentale in the uredinial and telial stages was collected in 1917 in 18 localities in Colorado and 5 in Arizona. Although all of these collections except those at Denver and Boulder, Colo., were made in regions where piñon pines occur, the Peridermium stage was collected only in 6 localities. *Ribes aureum* is the principal host for the uredinial and telial stages in nature and is usually heavily infected under favorable conditions, especially along streams and under moist conditions. At Glenwood Springs, Colo., *Grossularia leptantha*, growing on dry hillsides was abundantly infected, while *R. aureum* and *R. inebrians* were found infected in only one locality for each, and of the latter species only one clump or bush was infected. *R. odoratum* has been planted in many gardens in and around Prescott, Ariz. This species is doubtfully distinct from *R. aureum*, is equally susceptible to the fungus, and was abundantly infected at Prescott. Cultivated currants (*Ribes* spp.) and

[1] All collections from the District of Columbia are from artificial inoculations made in the greenhouse at Washington by Messrs. Hedgcock, Hunt, and Rhoads.

gooseberries were frequently found in Colorado in regions near badly diseased plants of *R. aureum*, but were never found infected. At Prescott a few plants of cultivated gooseberries (*Grossularia reclinata* × *G. hirtella*) were found to be slightly infected.

The apparent rarity of *Peridermium occidentale* is perhaps due chiefly to the fact that plants of susceptible Ribes are not of common occurrence in piñon forests. In localities at 8,000 feet or more in elevation, as in the San Luis Valley in southern Colorado, *Grossularia leptantha* is common among piñons, but lower down on the piñon mesas no species of Ribes are found except along the streams. Here *R. aureum* occurs frequently. In such localities agricultural activities have removed most of the trees of *Pinus edulis*, which formerly grew there in abundance.

The piñons of Colorado are all of the 2-leaved species, *Pinus edulis*, and are found almost exclusively west of the 106th Meridian and south of the 39th Parallel (fig. 1). They occur at an altitude of 5,000 feet to 8,500 feet, although in exceptional cases, as on Marshall Pass, they ascend to nearly 10,000 feet. They cover an area of more than 40,000 square miles of the State, chiefly the mesas (low table-lands) in isolated areas. Only a small part of this region was surveyed during 1917, owing to the lack of time and because of the difficulty of access, since there are few railroads or automobile roads in this section.

INOCULATIONS WITH CRONARTIUM OCCIDENTALE

INOCULATIONS WITH ÆCIOSPORES

The first inoculations with the æciospores of *Peridermium occidentale* from *Pinus edulis* were made at the pathological greenhouses at Washington, D. C., on June 1, 1917, with material (FP 24667) collected on May 25. Plants of *Castilleja linearifolia*, *Comandra umbellata*, and *Ribes odoratum* were used. On June 14 one of the plants of *R. odoratum* was found to be infected with a species of Cronartium. Two more *R. odoratum* plants were immediately inoculated with spores from the same lot of æcial material. On June 25 abundant uredinia were observed to be forming on several leaves of both plants. Successful inoculations were also made under rigid quarantine conditions in a unit of the quarantine house of the Federal Horticultural Board. Other æciospore inoculations were made at various times with material from different collections and localities. Inoculated plants of *R. aureum*, *R. odoratum*, and of *Grossularia inermis*, and one plant of *R. americanum* became infected, but those of *R. nigrum* and *G. missouriensis* remained uninfected. Control plants of all species were uninfected. The last successful æciospore inoculation was made on October 2, 1917, with material collected on September 21. No infections resulted where the æciospores used had been collected and kept for more than 20 days. Table I gives a summary of the results of the inoculations with æciospores and urediniospores at Washington, D. C.

TABLE I.—*Inoculations with æciospores and urediniospores of Cronartium occidentale at Washington, D. C.*

Host species.	Æciospore inoculations.			Urediniospore inoculations.		
	Plants inoculated.	Plants infected.	Plants uninfected.	Plants inoculated.	Plants infected.	Plants uninfected.
Ribes americanum [a]	4	1	3	9	5	4
Ribes aureum	6	3	3	8	4	4
Ribes coloradense	0	0	0	2	2	0
Ribes giraldi	0	0	0	1	1	0
Ribes glandulosum	1	0	1	2	1	1
Ribes malvaceum	0	0	0	2	1	1
Ribes nigrum [a]	15	0	15	22	2	20
Ribes odoratum [b]	32	16	16	45	28	17
Ribes sanguineum	0	0	0	1	0	1
Ribes sp. (?)	15	0	15	8	3	5
Grossularia inermis	10	3	7	10	5	5
Grossularia missouriensis	5	0	5	7	1	6
Grossularia reclinata [c]	0	0	0	1	0	1
Total	88	23	65	118	53	65

[a] Plants of *R. americanum* and *R. nigrum* inoculated with urediniospores were showered daily with spores for several days.
[b] *R. odoratum* was used almost exclusively when conditions were unfavorable; hence the percentage of uninfected plants of this species is misleading.
[c] The plants of *G. reclinata* were apparently a hybrid between this species and *G. hirtella*.

In addition to the inoculations made in the greenhouses at Washington, a number of plants of *Ribes aureum* were inoculated in the open at Denver and at Bayfield, Colo. Nearly all of these were successful, uredinia and telia being produced in abundance in most cases until in late October, when the heavy frosts came. Attempts to inoculate *R. americanum* and garden currants and gooseberries in the open at Denver and at Bayfield met with failure.

INOCULATIONS WITH UREDINIOSPORES

At Washington, D. C., inoculations with urediniospores were successful on *Ribes americanum*, *R. aureum*, *R. coloradense*, *R. giraldi*, *R. glandulosum*, *R. malvaceum*, *R. nigrum*, *R. odoratum*, *Ribes* sp., *Grossularia inermis*, *G. missouriensis*, and *G. reclinata* × *G. hirtella*. On plants of the *R. aureum* type many of the infected areas enlarge rapidly on the leaves, and the infection often spreads from leaf to leaf on the same and adjoining healthy plants. The uredinia in heavy infections are borne close together and eject spores in such masses that the leaf tissue appears to be covered. On plants of *R. malvaceum*, of *G. inermis*, and of *G. missouriensis* infected areas enlarge rather slowly and do not usually produce uredinia or spores in abundance. The fungus sometimes spreads to new areas of diseased leaves and even to new leaves on infected plants of *G. inermis*, but has not been known to do so on plants of *R. malvaceum* or of *G. missouriensis*. No plants of *R. americanum* or *R. nigrum*

became infected until late in the summer, although many inoculations were attempted. On plants of these two species the infected areas developed slowly until they became from 2 to 4 mm. in diameter. The invaded tissues often die soon after infection. Uredinia are scanty if produced at all, and few spores develop. The incubation period for these two species may be as long as 36 days, although 15 days are the maximum for other species. The disease has not been observed to spread to new areas or to new leaves on plants of these species.

The leaves of many plants were carefully examined with a hand lens almost daily, beginning 5 or 6 days after the date of inoculation. In no case were uredinia found forming in less than 10 days following inoculations with æciospores, nor in less than 9 days following inoculations with urediniospores. Urediniospores are normally produced in 12 to 15 days after inoculations with either form of spores, except as noted above for *R. americanum* and *R. nigrum*.

A summary of inoculations with urediniospores during 1917 and the spring of 1918 at Washington, D. C., is given in Table I. Urediniospores and teliospores were produced throughout the winter, successive inoculations being made every six weeks.

INOCULATIONS WITH TELIOSPORES

The first inoculations with the telia of *Cronartium occidentale* from *Ribes aureum* sent in from Colorado were made in 1914. Others were made in 1916. The material used in these inoculations was in such condition that probably no viable teliospores were present, as no results have been obtained. In 1917 about 4,000 inoculations were made on 23 species of pine. The larger part of these were made in the field, in Colorado and Arizona, but a considerable number were made in the greenhouse at Washington, D. C. It is hoped that some indication of the results may be obtained during 1918 either through the development of pycnia or by determining the presence of the mycelium of the species of Cronartium by Colley's method (*3*).

DISSEMINATION OF CRONARTIUM OCCIDENTALE

Cronartium occidentale, like other species of the genus Cronartium in the United States (*4*, *6*), is disseminated in three spore forms: Æciospores, urediniospores, and teliospores.

Although the æcia of the Peridermium form of this fungus on *Pinus edulis* were first found in the fruiting stage on May 25, they may sometimes mature at a somewhat earlier date. Viable spores of the Peridermium were found in specimens collected in southern Colorado as late as September 21. The æciospores are discharged slowly, owing to the fact that they are held under the bark in large flattened cavities which rupture by means of narrow rifts in the bark. In 1917 the æciospores were not found infecting *Ribes* spp. at a greater distance than a hundred

yards; but the season was dry, and this factor may have limited the area of infection.

The uredinial stage (Pl. 57) of the rust on species of Ribes follows the infection by æciospores at the end of 12 to 15 days, or in cool weather a few days longer. In nature the reinfection of the species of Ribes by urediniospores may follow in about two weeks, the time being largely influenced by physical conditions, thus continuing the reinfection until the end of the growing season. It is not uncommon to find an abundance of uredinia on *R. aureum* and *R. odoratum*, with viable urediniospores in late autumn, even after freezing weather begins. Both the æciopores and unrediniospores undoubtedly are chiefly wind-disseminated. Insects may carry them to some extent. Cattle, sheep, and goats feed on *R. aureum*, brushing against the diseased leaves. During moist weather they are probably instrumental in spreading the disease.

The telial stage (Pl. 57) in nature ordinarily follows the uredinial stage in one to two weeks, varying somewhat with the temperature of the air and the age of the infected leaves, the controlling conditions not being well known. The mature teliospores germinate *in situ*, the telia assuming a silvery tinge, owing to the presence of sporidia. Pines in turn are infected by the sporidia, the pycnial and æcial stages following in succession after a period of one or probably more years, the time probably varying as in other species of Cronartium (*4*, *6*).

Cronartium occidentale seems to be able to overwinter on *Ribes aureum*. This is indicated by collections of the disease by one of the writers (Bethel) from the same clumps of *R. aureum*, and even from the same bushes, year after year in the parks of Denver and Boulder. The first collections of the disease at these places were made in 1909. It was subsequently found in the same places each year until 1917, when a severe drouth seems to have killed the organism in all of the clumps on which the disease had previously been found. The only known natural occurrence of the disease in either city during 1917 was a scattering infection found by Mr. Hunt in a group of plants of *R. aureum* in Washington Park, Denver. Conditions were such that it is quite possible the disease will not reappear there. It seems inconceivable that æciospores could be blown 50 miles or more and infect the same plant or groups of plants year after year, while many plantations near by remained free from infection.

Æcial infections usually occur on more or less scattered leaves all over the plants infected, and not on a single well-protected branch in the center of a clump. The greater part of the collections of the uredinial and telial stages of the fungus in 1917 were made in localities too remote from piñons for any probable æcial infection, and the manner of development indicated that the rust had in some way lived over the winter on the plants. In a few cases remote from piñons in 1917 the uredinial stage was observed at first on a single well-protected branch of a large

clump of *Ribes* sp. growing in a moderately damp place. Uredinia and telia are common on the petioles of the leaves and are occasionally found on the flower peduncles, but so far have not been observed on the stems. Posey, Gravatt, and Colley (5) have, in the case of *Cronartium ribicola*, found natural stem infections of *Grossularia hirtella*, which bore normal urediniospores. *Melampsora bigelowii* Thüm., also a common rust in Colorado, is known to overwinter on stems on *Salix* spp. here and elsewhere.

During a succession of seasons favorable for the spread and overwintering of *Cronartium occidentale* it might be distributed in the uredinial stage to regions far distant from piñon pines. *Coleosporium solidaginis* (Schw.) Thüm. on species of Aster and Solidago, which has for its æcial stage *Peridermium acicolum* Underw. and Earle, a needle-rust of pines, spreads across the plains for long distances in this way. *Coleosporium ribicola* (C. and E.) Arth. on species of Grossulariaceae is found in Montana nearly 400 miles to the north of the range of *Pinus edulis*, its only known natural æcial host. The occurrence of *Cronartium occidentale* at Stockton, Kans., in 1892, as reported by Arthur (2) may be explained similarly. Its occurence sporadically in the parks of Denver and Boulder may have been the result of large plantings with stock of *Ribes aureum* shipped from Colorado points where the disease is epidemic. Shipments are known to have been made from Rifle, where the disease was especially abundant in 1917.

In the spring of 1917 many infected leaves in a good state of preservation, bearing urediniosori which still had their natural color, were collected in the open at Denver and Bayfield, Colo., and used in inoculation experiments at Washington, D. C., without positive results. Attempts to germinate the urediniospores at the latter place failed also. The possibility that such urediniospores may remain viable over the winter should be investigated more thoroughly.

EFFECT OF THE FUNGUS ON ITS HOSTS

Peridermium occidentale is rarely found on very old trees of *Pinus edulis*, and in these it is in the crevices of the bark often with no adjacent dead areas. In such cases even when fruiting it is hardly discernible with a hand lens. Its effect on young trees is more apparent. A number of young trees apparently killed by the fungus were found in southern Colorado. Such trees are usually attacked on the trunk and branches near the ground. Some become spike-topped (Pl. 56). Lesions on old trees usually are found from 2 to 8 feet from the ground.

Plants of species of Ribes and Grossularia attacked by *Cronartium occidentale* apparently suffer but slight injury, owing to the fact that the season's growth is made chiefly before the infection becomes heavy. In severe early attacks partial defoliation may result, usually late in the growing season. Repeated attacks may stunt somewhat the growth of

the plants, but none have been found killed from the effects of the fungus. On leaves attacked by the fungus the uredinia and telia cause a characteristic spotting. The spots vary in color from a light yellow-green before frost occurs to a purplish brown afterwards. This spotting is most pronounced on leaves of *R. aureum* and *R. odoratum.*

SUMMARY

The species of Cronartium native on *Ribes* spp. and *Grossularia* spp. in Colorado and Arizona is described for the first time and named "*Cronartium occidentale.*"

The æcial stage of this fungus is proved to be a form of Peridermium on the piñons, or nut pines, *Pinus edulis* and *P. monophylla*, and is now given the form-name "*Peridermium occidentale.*"

Cronartium occidentale is widespread throughout the piñon region of Colorado, extending into Arizona. Further surveys will no doubt greatly extend the known range of the species.

The common native host for the telial stage of this fungus is *Ribes aureum*, although it occurs occasionally on *R. odoratum*, *R. inebrians*, and *Grossularia leptantha.*

Cronartium occidentale has been successfully inoculated on *Ribes americanum*, *R. aureum*, *R. coloradense*, *R. giraldi*, *R. malvaceum*, *R. nigrum*, *R. glandulosum*, *R. sanguineum*, *Ribes* sp., *Grossularia inermis*, *G. missouriensis*, and *G. reclinata* × *G. hirtella.*

Peridermium occidentale, so far as known, attacks ony the piñon pines. Inoculations have been made on 23 species of pines to ascertain whether it will attack other kinds of pines.

Cronartium occidentale differs essentially from *C. ribicola*, cause of the white-pine blister-rust, especially in the æcial stage. A synopsis of these variations is given.

Cronartium occidentale is apparently able to overwinter and maintain itself independent of the æcial stage. Only circumstantial evidence in support of this view has been obtained.

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PLATE 54

Specimens from the type collection of the æcial stage (*Peridermium occidentale*) of *Cronartium occidentale* on *Pinus edulis* from Bayfield, Colo. One twig has an æcial cavity exposed by turning back the overlying bark.

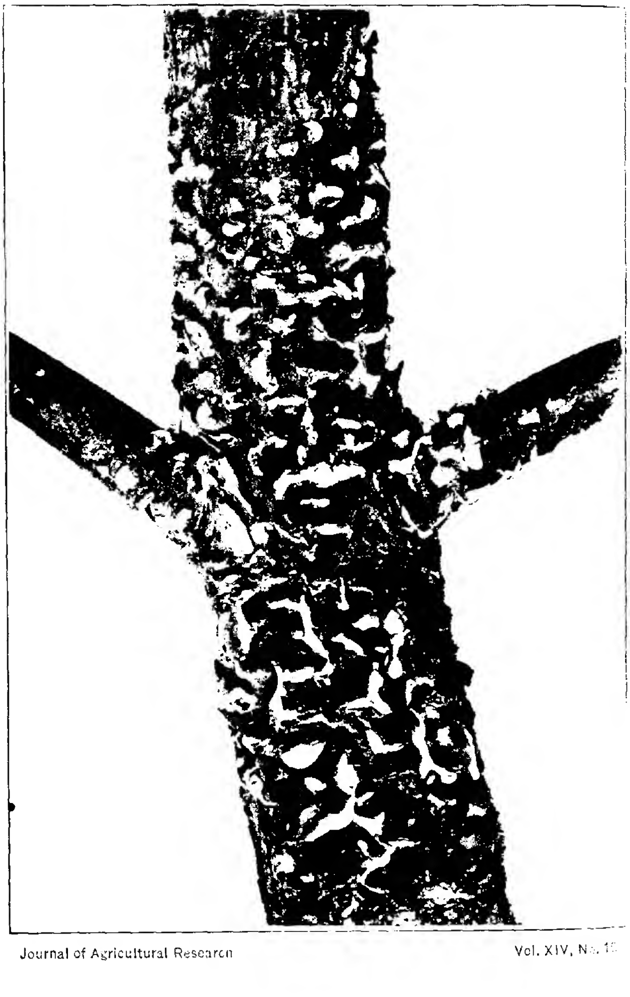

PLATE 55

The æcial stage (*Peridermium strobi*) of *Cronartium ribicola* on *Pinus strobus* from Kittery Point, Me. (Photographed by Dr. R. H. Colley).

PLATE 56

A young piñon tree (*Pinus edulis*) diseased with *Peridermium occidentale* in the pycnial stage, natural infection. Transferred from the forest near Mancos, Colo., to a pot in the greenhouse at Washington, D. C. The top has been killed by the fungus, giving it a spindle form, such as does not occur in trees fully alive.

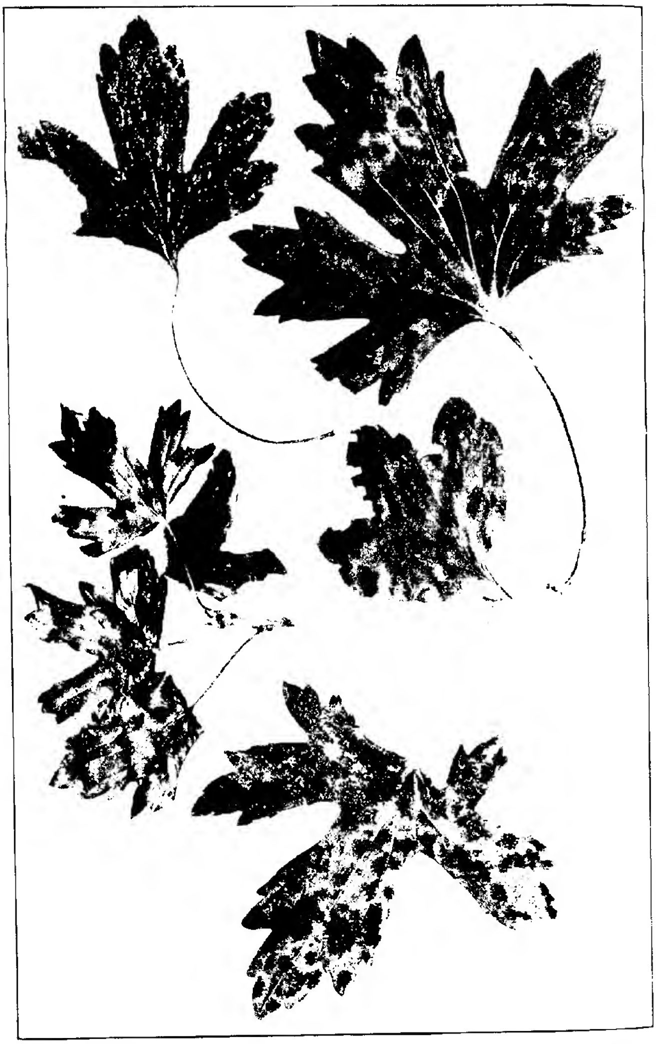

PLATE 57

The uredinial and telial stages of *Cronartium occidentale* on *Ribes aureum* from the type material collected at Bayfield, Colo.; artificial inoculation.

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COMPARATIVE TOXICITY OF COTTONSEED PRODUCTS

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INTRODUCTION

Notwithstanding the isolation by us of a distinctly toxic substance (gossypol) from cottonseed (*15*),[1] six papers have recently appeared offering to explain cottonseed-meal poisoning or "injury" on the theory of dietary deficiencies. The chief support for this view is found (a) by Rommel and Vedder (*13*) who fed pigs on polished rice and tankage developed symptoms similar to those fed at the same time on corn meal and cottonseed meal, and (b) by Richardson and Green (*10, 11, 12*) and by Osborne and Mendel (*7, 8*), who show that, while white rats will ultimately fail on diets containing cottonseed meal as the sole source of protein, minerals, and vitamines, the animals will grow normally on cottonseed-meal or cottonseed-flour diets if supplemented by milk powder, protein-free milk, butter, etc.

Wells and Ewing (*14*) have also adopted the dietary-deficiency view as being the most plausible explanation of cottonseed-meal injury. They base their conclusion that cottonseed meal is an incomplete food on experiments with very young pigs confined in metabolism cages, the cottonseed ration being supplemented with starch, sugar, and small quantities of milk. In some cases they admit that the injury may have been due to the presence of a toxic substance.

The fallacy in concluding, from the ultimate failure of rats on diets in which cottonseed meal is the sole source of protein, minerals, and vitamines, that dietary deficiencies are the cause of cottonseed-meal injury in swine, for example, is evident if we base a somewhat similar argument as to other seeds on the results of feeding them to rats as the sole source of these nutritive factors. For example, rats similarly restricted to any seed will show failure, and in most cases much sooner than on cottonseed meal, but we know that in the diet of swine these seed diets do not cause the sudden death which is typical of cottonseed-meal injury. Another distinct difference between the failure of rats on restricted cottonseed-meal diets and the failure of swine on cottonseed meal under farm conditions is that with rats death usually follows a period of low food intake and consequent emaciation, while with swine it is very well known that death often follows a period of excellent growth and with the animals in splendid nutrition. None of these investigators has fed

[1] Reference is made by number (italic) to "Literature cited," p. 451-452.

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diets designed to reveal whether the failure of their animals was due to inadequate diets or to the presence of a mildly toxic substance. We believe we have accomplished this by comparing ether-extracted cottonseed kernels with cottonseed meal.

After the appearance of Rommel and Vedder's (*13*) preliminary note, we conducted some preliminary experiments with pigs (*16*) in which we found gossypol isolated from raw cottonseed to be markedly toxic to these animals and also demonstrated that while animals on cottonseed meal quickly sickened and died, pigs which were fed on the ether-extracted cottonseed kernels were not affected. This fact seems to us to outweigh the hypothesis advanced regarding deficient diets as the cause of cottonseed-meal injury, and to confirm our previous view. With reference to the presence of a deleterious substance in cottonseed meal, we would emphasize at the outset the fact that pigs, rats, and rabbits grow better on a diet containing ether-extracted kernels than on a diet containing cottonseed meal. Pigs and rabbits are so quickly affected by cottonseed meal that we were led to state (*15*) that—

> generally speaking, the meal and the kernels are toxic to the same degree.

Many subsequent experiments by us with four species of animals have shown that meal is far less toxic than kernels for rats and hens, but the fact remains that cottonseed meal, even a thoroughly cooked meal, is highly injurious to rabbits and pigs.

Following the paper by Richardson and Green (*10*) we fed rats on cottonseed products. The vast difference between the highly toxic raw seed and the very slightly toxic cooked product was immediately evident. This indicated to us that in the manufacture of cottonseed meal the gossypol undergoes some change whereby the meal is rendered much less toxic than the original kernels.

In this paper we report some of the experiments conducted to ascertain to what extent the change in toxicity takes place under oil-mill conditions. These experiments led to the conclusion that there still remained a toxic factor in all the samples of cottonseed meal and cottonseed flour which we fed. Rats and hens are less affected by this factor than rabbits and swine. In fact, in diets well supplemented with milk powder the toxic factor for rats may remain entirely masked.

OIL-MILL TREATMENT OF COTTONSEED PRODUCTS

In order to compare the effects of cooking on the toxicity of cottonseed, we have used products obtained from cottonseed-oil mills. For comparing the effect on different species, two meals were extensively fed. These, together with other samples, were collected by one of us (Carruth) personally at the oil mills. Information was also obtained regarding the length of time, steam pressure, and other details of the cooking process. It is evident to us that the

"home-made" products of Osborne and Mendel do not resemble mill products. For example, their raw kernels "subjected to vigorous treatment with live steam from one to six hours," in which there was some loss through distillation and spraying, underwent a change in toxicity much more slowly than in the commercial process. In particular, their laboratory treatment of the kernels made the mass so wet that it had to be dried before feeding. This is not the condition in the oil mill, where air is often drawn through the cooking drum to remove excess moisture. Under mill conditions the kernels undergo a much more rapid reduction in toxicity, for we have found oil-mill samples after 25 to 28 minutes' cooking not markedly injurious to rats in adequate diets, while Osborne and Mendel's (*8*) product cooked for one hour was found by them to be appreciably toxic. After four hours' cooking under laboratory conditions their product became less suitable for nutrition, and the results led them to assume that undue heating might render the meal unpalatable. This also seems to us unlikely to happen in the better regulated conditions of moisture and heating in the industry.

Another point of marked difference is the fact that their actually cold-pressed oil was nontoxic, whereas we have found that the commercial so-called cold-pressed [1] oil is highly toxic because most of the gossypol from the resin glands has been dissolved by the oil and squeezed out unchanged.

Our experiments (*16*) with raw kernels and gossypol led us to believe that gossypol is the only toxic substance in the raw cottonseed. The toxicity of the cottonseed meal varies with the amount of unchanged gossypol present. But from certain meals which we found definitely injurious to rabbits and swine no gossypol could be isolated by our methods. Apparently it had been entirely changed in the cooking process to a very similar substance, called by us "D-gossypol," which is much less toxic but which can not be regarded as being without physiological action.

In the milling of cottonseed the decorticated kernels are cooked in steam-jacketed drums while being continually stirred with huge paddles. The mass becomes somewhat moist as the temperature rises, either from moisture present in the seed or from steam sprayed in, if the seeds are too dry. In this condition the kernels are quickly comminuted. These conditions are favorable for effecting an important change in the gossypol which issues from the glands and then spreads over the surface of the seed particles. The nature of

[1] The "cold-pressed" meal is made from kernels passed through preheaters surrounded by steam under pressure. This serves to drive out the moisture. The dried kernels are then ground in a screw press to express the oil. This also develops much heat from friction. The actual temperatures used in the hot pressing and cold pressing do not differ greatly. The latter is really a dry pressing rather than a moist pressing.

this change has not been ascertained, but it seems plausible to suppose it is an oxidation process.[1] The gossypol appears to be converted into D-gossypol, which is very similar in color reactions to gossypol, but which is much less soluble and has a higher melting point. In the hot-pressing process the highly toxic gossypol, under favorable conditions, is quickly changed to this considerably less toxic substance.

COOKING PROCESS

The two meals which we used so extensively may be designated as the "short-cooked" meal and the "long-cooked" meal. The short-cooked meal was obtained from an oil mill using single "cookers," or steam-jacketed drums. The raw kernels after decortication and crushing are conveyed to the drum, where they are stirred by large revolving paddles. The steam pressure in the jackets was about 40 pounds. The kernels themselves are not subjected to steam pressure. In this drum the kernels are cooked for 28 minutes; then the drum is emptied; and the kernels are ready for the "cake former." In the meantime they are kept hot. The cake former allows a definite amount to pass out onto haircloth mats. The mat is folded and molded by light pressure and then carried to the hydraulic press. When enough cakes have been molded, pressure is exerted and maintained until the oil ceases to drip. The hard cake is then removed, usually allowed to cool, and then ground into the meal of commerce. From this particular mill sufficient meal was obtained for the experiments with rats, rabbits, fowls, and swine. In order to follow the change in toxicity on cooking, we took samples from the cooking drum at 5-, 10-, and 20-minute periods and also a sample of the kernels cooked the full period—28 minutes in this case. These products were all fed to rats as described on pages 431–431 (see fig. 1). A sample of oil from these fully cooked kernels was also obtained; however, as it contained practically no gossypol, it was not fed, but was assumed to be nontoxic, as was a similar sample secured from this mill at another time.

The long-cooked meal was obtained from a mill using a 5-"stack" (or drum) "French continuous cooker." In this type the steam-jacketed drums are placed one over another. The kernels enter the top drum and pass down as the drum below is emptied. In this mill the cooking operation consumed about two hours. The kernels and meal were considerably darker than the short-cooked meal and would be considered as off color, the meal being almost reddish brown instead of the usually highly regarded "bright-yellow" meal. Samples of fully cooked kernels and oil

[1] It is possible that this change may be otherwise effected in the unknown conditions of the colloidal material. Our reason for believing that it is not due to heat alone is that B-gossypol obtained by heating gossypol to 180° C. differs in properties from D-gossypol. This temperature is about 80° higher than the temperature of cooking cottonseed in the manufacture of cottonseed meal.

therefrom were also secured. The steam pressures in the jackets surrounding the various drums ranged from 20 to 40 pounds.

Since this long cooking period represents the upper limit of time and thoroughness of cooking in the cottonseed industry, the results of our rabbit and swine experiments especially are highly significant, in view of the claims that cottonseed-meal injury is a matter of dietary deficiency,

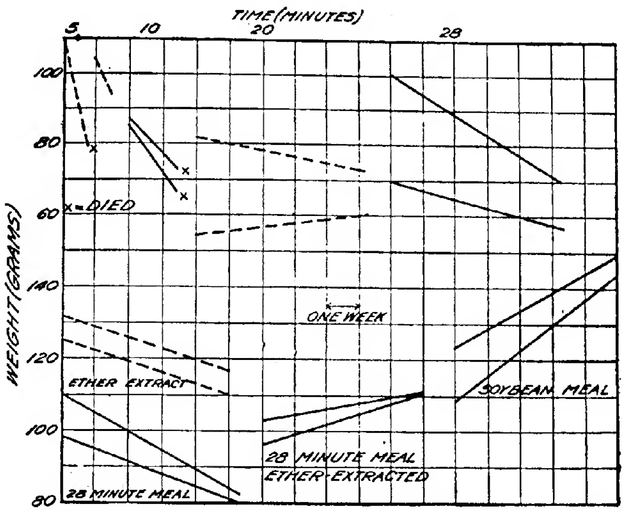

FIG. 1.—Graphs showing the toxicity of cottonseed meal and kernels to rats. The upper curves represent the records of rats fed cottonseed kernels cooked various lengths of time in a commercial oil mill. A marked change in toxicity occurs between the 10- and 20-minute interval. The lower curves show the effect of the meal made from the kernels cooked for 28 minutes and also the beneficial effect of ether extraction. The record of the rats fed soybean meal shows what growth might be expected if the diets had been free from toxic substances. The composition of the diets is given on page 431.

and with reference to the conclusion by Osborne and Mendel (*8*) from the results of their rat experiments that the—

> treatment of cottonseed so as at least to render it harmless now seems to lie within the range of ready possibilities.

A sample of the long-cooked meal used in the pig diets, also in the rabbit, rat, and poultry diets, was sent to Dr. T. B. Osborne, at the Connecticut Agricultural Experiment Station, who wrote as follows:

> Our results with the cottonseed meal from the French cooker show that this treatment has apparently entirely destroyed the toxic properties of the seed. All of our rats on this product are growing finely.

In these experiments Dr. Osborne supplemented the meal with protein-free milk and butter fat. It is evident that the results with rats do not indicate what effect meal has on rabbits and pigs.

EXPERIMENTAL WORK

EXPERIMENTS WITH RATS

On cottonseed meals which contain no unchanged gossypol, but apparently only the D-gossypol, rats have not done so well as on ether-extracted raw kernels which contain no D-gossypol and only traces of unextracted gossypol. (See Table I, diets 390, 392, 393, 383, and 383A; see also fig. 2, 3.) This fact leads us to maintain that there is still a toxic factor in well-cooked cottonseed meals. The difference holds true of the restricted cottonseed diets, which are practically identical with respect to protein, minerals, and vitamines. When these products are supplemented with 17 per cent of milk powder, there is no

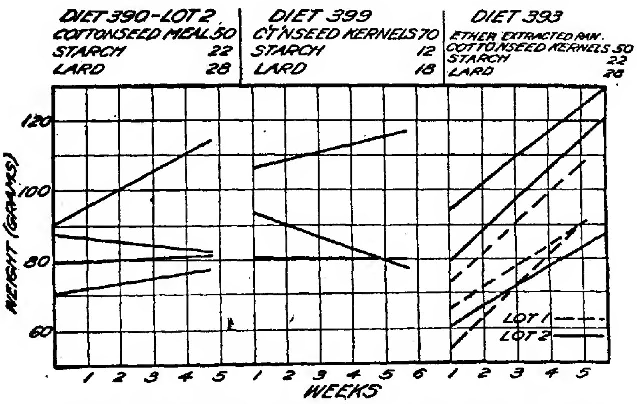

FIG. 2.—Graphs showing the toxicity of various diets to rats. Thoroughly cooked cottonseed products, diets 390 and 399, are inferior to ether-extracted raw kernels, diet 393, in restricted diets, apparently because there remains a quantity of a moderately toxic substance, D-gossypol, in the cooked products. This difference is not as noticeable in diets supplemented with milk powder or with certain mineral salts and butter fat. All these diets finally led to absolute failure.

visible difference; in fact, such diets appear as efficient as the control milk diet for rats. In this respect our results agree with those of Osborne and Mendel (*8*) and Richardson and Green (*10*, *11*, *12*).

The rats used were bought from a dealer and were apparently inferior animals both in size and vitality. Few of them exceeded 200 gm. in weight, and many ceased growing at 100 to 125 gm. In the experiments animals from the same shipment were used in one set so that the results are generally but not always comparable with the results of other sets. In only one case out of many experiments with rats on unsupplemented cottonseed meal (long-cooked) diets have our rats shown appreciable growth. This instance (feed 390, lot 5) was with two rats from a litter reared in our animal rooms. The mother and young were fed on the control milk diet with occasional green food until

the young were 55 days old. During the subsequent 31 days these two rats made excellent gains; in fact, they grew nearly as well as the rest of the litter on the milk diet. It is quite possible that the previous diet may explain why they made such a better record. It is well-known that rats supplied by dealers are often less vigorous, probably owing to the fact that often they have been reared on insufficient diets.

TABLE I.—*Effect of various diets on the growth of rats*

UNSUPPLEMENTED DIETS

Experiment and diet No.	Number of rats.	Diet.	Average weight. Initial.	Average weight. Final.	Change.	Duration of experiment.
			Gm.	*Gm.*	*P. ct.*	
EXPERIMENT 1:						
390, lot 1	3	Cottonseed meal, 50 per cent.	52			Died in 9, 17, and 47 days.
392	3	Cottonseed flour, 50 per cent.	50			Died in 9, 16, and 47 days.
393, lot 1	3	Ether-extracted cottonseed kernels, 50 per cent.	65	99	+52	Discontinued after 29 days.
385	3	Soybean meal, 50 per cent; crude cottonseed oil, 14 per cent.	70	85	+21	Discontinued after 22 days.
EXPERIMENT 2:						
399	3	Cottonseed kernels cooked 2 hours, 70 per cent.	94	91	− 3	Discontinued after 33 days.
390, lot 2	4	Cottonseed meal from above kernels, 50 per cent.	82	89	+ 8	Do.
393, lot 2	3	Ether-extracted cottonseed kernels, 50 per cent.	78	112	+44	Do.
EXPERIMENT 3:						
390, lot 5 (see p. 430).	2	Cottonseed meal as in experiment 2, 50 per cent.	101	143	+42	Discontinued after 31 days.
Do	2	Milk diet (control)	89	136	+53	Do.

SUPPLEMENTED DIETS [a]

Experiment and diet No.	Number of rats.	Diet.	Average weight. Initial.	Average weight. Final.	Change.	Duration of experiment.
EXPERIMENT 4:						
424	1	Cottonseed kernels cooked 5 minutes.	109	78	−28	Died in 5 days.
425	2	Cottonseed kernels cooked 10 minutes.	86	71	−18	Died in 9 days.
426	2	Cottonseed kernels cooked 20 minutes.	68	68	− 0	Discontinued after 37 days.
427	2	Cottonseed kernels cooked 28 minutes.	85	65	−23	Do.

[a] Diets 424 to 427, inclusive, contained 70 per cent of cottonseed kernels, 12 per cent of starch, and 18 per cent of lard. Diets 428 to 433 contained 50 per cent of meal, 22 per cent of starch, and 28 per cent of lard. The cottonseed kernels used in diets 424, 425, 426, and 427 contained, respectively 0.62, 0.24, 0.10, and 0.07 per cent of unchanged gossypol.

TABLE I—*Effects of various diets on the growth of rats*—Continued

UNSUPPLEMENTED DIETS—continued

Experiment and diet No.	Number of rats.	Diet.	Average weight. Initial.	Average weight. Final.	Change.	Duration of experiment.
EXPERIMENT 4—Continued.			*Gm.*	*Gm.*	*P. ct.*	
428	2	Cottonseed meal made from kernels cooked 28 minutes.	104	80	−23	Do.
429	2	"Buco" cottonseed meal cooked (?) minutes.	66	73	+11	Discontinued after 38 days.
432	2	28-minute cottonseed meal, ether-extracted.	99	111	+12	Discontinued after 35 days.
431	2	Ether extract of 28-minute cottonseed meal, 12 per cent.	129	114	−12	Do.
433	2	Soybean meal	116	147	+27	Do.
435 [a]	6	28-minute cottonseed meal, 45 per cent; whole milk powder, 17 per cent.	71	114	+61	Discontinued after 29 days.
EXPERIMENT 5:						
372	3	Ether extract of cottonseed flour, 28 per cent.	82	76	− 7	Discontinued after 10 days.
372A		Ether extract of cottonseed flour, 7 per cent.	76	106	+39	Discontinued after 21 days.
372B		Ether extract of cottonseed flour, 10 per cent.	106	124	+17	Do.
388	2	Petroleum-ether extract of cottonseed kernels, 14 per cent.	199	208	+ 4	Discontinued after 40 days.
388A		Kernels extracted by petroleum ether.	130	119	− 8	Discontinued after 3 days. Refused to eat.
440	2	Crude cold-pressed cottonseed oil, 14 per cent (gossypol 0.21 per cent).	115	81	−30	Discontinued after 14 days. Fatal to one.
438	2	Cold-pressed cake meal (mill M), 40 per cent with milk powder.	105	111	+ 6	Discontinued after 10 days.
438A		Cold-pressed cake meal (mill M), 50 per cent, with starch and lard only.	110	93	−15	Do.
439	2	Cold-pressed meal from mill E, 50 per cent.	110	108	− 2	Discontinued after 8 days.

[a] The rats on diet 435 were the emaciated animals previously on diets 426, 427, and 428.

Table I—*Effects of various diets on the growth of rats*—Continued

UNSUPPLEMENTED DIETS—continued

Experiment and diet No.	Number of rats.	Diet.	Average weight. Initial.	Average weight. Final.	Change.	Duration of experiment.
Experiment 5—Continued.			*Gm.*	*Gm.*	*P. ct.*	
451	2	Cold-pressed meal from mill W, 50 per cent.	148	152	+ 3	Discontinued after 15 days.
[a] 460	2	Hot pressed meal from mill T, 50 per cent.	126	109	−13	Discontinued after 14 days.
460A	3	Ether extract of above 12.5 per cent, with milk powder.	143	116	−17	Discontinued after 10 days.
Experiment 6:						
380	3	Soybean meal, 70 per cent; lard, 30 per cent.	45	51	+13	Discontinued after 79 days. Failure.
381	3	Soybean meal, 25 per cent; corn meal, 75 per cent.	46	47	−2	Discontinued after 30-40 days. Failure.
375	3	Cottonseed meal, 25 per cent; corn meal, 75 per cent.	46	37	−20	Discontinued after 50 days. Failure.
383	3	Period 1: Cottonseed flour, 30 per cent; corn meal, 42 per cent; lard, 28 per cent.	67	79	+18	Discontinued after 68 days. Stopped growing entirely.
383A		Period 2: Ether-extracted cottonseed kernels in place of cottonseed flour (fig. 3.)	79	106	+34	Discontinued after 68 days; better growth than during period 1.

[a] The meal in diet 460 contained some unchanged gossypol.

In experiment 1 the rats on the extracted kernels made fair progress until failure set in after 140 days. Rats on soybean meal failed after 128 days. These diets contained 50 per cent of the food under experiment, together with 22 per cent of starch and 20 per cent of lard.

In attempts to find a cottonseed meal which would be markedly toxic to rats, even in milk-powder diets, we fed several samples of so-called cold-pressed meal. Here, also, we failed to find marked toxicity. This was puzzling at the time, but investigation showed that in the so-called cold-pressing a large amount of the gossypol passes into the crude oil, which is highly toxic, and the remainder is more or less changed, so that there is nothing inconsistent in these results. It may be mentioned that by actually using cold pressure on raw cottonseed kernels, Osborne and Mendel obtained an oil which was not appreciably toxic, though the

resultant meal was highly toxic. We have shown practically the same thing by feeding kernels and oil extracted by petroleum ether (table I, diets 388 and 388A).

It would seem that by the extraction with ethyl ether of certain cottonseed meals and flour, a small amount of toxic substance is removed. Thus, 28 per cent of the oil extracted from the flour was unpalatable to rats in a milk-powder diet; but when reduced to 7 and 10 per cent, it failed to have any noticeable influence (compare diets 372, A and B, Table I). In diets 428, 431, and 432 the ether-extracted meal was less active (fig. 1).

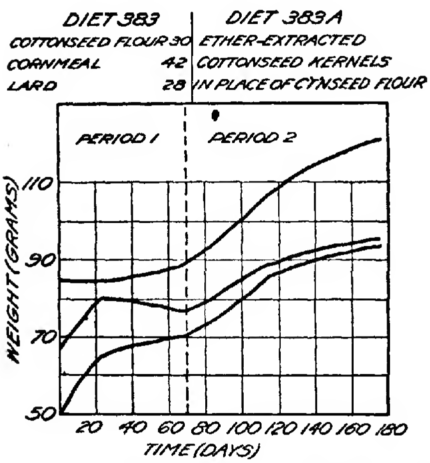

FIG. 3.—Graphs showing the toxicity of cottonseed flour to rats. They practically ceased growing after 68 days. A renewed growth impetus was shown when ether-extracted raw kernels were substituted for the flour. The flour contains the moderately toxic substance D-gossypol.

It should be noted here that cottonseed meal is not rendered nontoxic for rabbits (see diet 411, Table II) by ethyl-ether extraction, a fact which seems in harmony with the fact that in the extracted meal there is left a substance which responds to the tests for gossypol. The incomplete removal of this material thus lessens the decisive value of experiments with extracts from cottonseed meal.

Three samples of crude cottonseed oil prepared by hot pressing did not prove active in short feeding experiments. Tests made previous to feeding showed only traces of gossypol, and hence led us to predict this result. On the other hand, we found that in the commercial cold-pressing process most of the gossypol passed into the crude oil. This oil, which contains at least 1.5 per cent of gossypol, was found to be highly toxic (see diet 440, Table I, commercial cold pressed). The meal was not more active than short-cooked cottonseed meal (compare diets 439 and 451, Table I.)

Except in rare instances, cottonseed meal is fed in combination with other feeds. It has been found at this Station that steers fed exclusively on cottonseed meal and hulls do not thrive as well as where the roughage is silage. The meal and hulls in combination may not be fed safely for more than 70 to 90 days.

With pigs from 10 to 50 per cent of cottonseed meal has been fed. The usual experimental diet at this Station has been cottonseed meal 25 parts, corn or corn meal 75 parts. This diet soon causes pigs to diminish their food intake, and serious results will ensue if the pigs are kept on this ration. Deaths occur frequently after five weeks. Undoubtedly, even with no toxic factor present, a severely restricted diet of this type, as corn with other protein supplements, such as soybean meal, peanut meal, etc., is inadequate for normal growth and reproduction of pigs. It would appear from the insufficiency of a vegetarian diet that rats would also fail to make normal growth in diets of this type; compare Slonaker (*6*) and McCollum et al. (*5*, *6*).

A few experiments with rats have confirmed this opinion, and from the improvement of animals when given salts and butter fat it would appear that rations of this type contain an unsuitable inorganic basis and insufficient amounts of the fat-soluble A. This seems to be in harmony with our knowledge of the common seeds which have been studied by McCollum and his associates.

Thus, young rats on cottonseed meal and corn meal (1:3) failed to grow and ultimately died, but not sooner than rats on soybean meal and corn meal fed in the same proportion. Some older rats maintained their weight for 40 days, but also finally failed.

The addition of calcium, sodium, and chlorin, elements in which the ash is very poor, and the addition of butter proved beneficial after some of the survivors had begun to decline.

These experiments with soybean meal were conducted to aid in the interpretation of the cottonseed experiments. The percentage of protein and the inorganic content of soybean meal are very similar to that of cottonseed meal.

Experiments (unpublished) with pigs by the Animal Industry Division of this Station have shown that soybean meal is vastly superior to cottonseed meal for pigs; still, when the animals are confined to pens and restricted to corn and soybean meal, the appetite is impaired after several months, and a sort of pathological lameness and stiffness of the legs appears.

The facts stated above show that these protein concentrates in restricted diets may ultimately cause failure. This phenomenon is, in our experience, entirely distinct from the sudden death of pigs on cottonseed meal, which may follow excellent growth.

EXPERIMENTS WITH RABBITS

Rabbits have been used extensively for experimental feeding of cottonseed products at this Station. The general methods of feeding were described in our previous article (*16*). Under our conditions young rabbits grew rapidly and grown rabbits fattened or maintained weight on

control diets of corn meal or soybean meal and molasses instead of cottonseed meal. On cottonseed products the rabbits showed varying behavior, from being made sick (diarrhea) on one or two doses of raw kernels to consuming the ether-extracted kernels without noticeable harm for 75 to 200 days. Toward various cottonseed meals the rabbits also showed varying reactions. Some meals not thoroughly cooked were refused in less than a week, while other meals were eaten for more than three or four weeks before the rabbits became sick. The same general results were observed in these as in the rat experiments. The ether-extracted raw kernels were least toxic of all, but the rabbits were rather sensitive even to them, and occasionally became sick after long feeding periods when fed at the rate of 1 per cent of body weight daily, the usual rate of feeding. This is apparently due to incomplete extraction of the kernels (Table II).

TABLE II.—*Effect of various diets on the growth of rabbits*

Diet No.	Number of rabbits.	Food.	Average weight. Initial.	Average weight. Final.	Change.	Percentage food eaten is of initial weight.	Duration and result.
			Gm.	*Gm.*	*Gm.*		
325	6	Raw cottonseed kernels.[a]	1,620	1,477	−143	2.7	Discontinued after 6–26 days. Refused to eat much. Made sick after 10–20 gm. were eaten.
349	4	.....do..............	2,332	2,000	−332	3.8	Discontinued after 15–42 days. Refused to eat.
350	7	Ether-extracted cottonseed kernels.[a-b]	1,620	2,025	+405	75.0	Discontinued after 73–204 days. Occasionally went off-feed.
...	[c]3	Cottonseed flour.....	455	527	+ 72	9	All died in 12 days.
350	[c]3	Ether-extracted cottonseed kernels.	392	527	+135	20	Discontinued after 16 days. Not affected.
407	3	Soybean meal.......	1,015	1,515	+500	69	Discontinued after 57 days. In normal health.
406	3	Long-cooked cottonseed meal, No. 1.	1,038	1,033	− 5	26	Died in 21–40 days.
410	3	Short-cooked cottonseed meal.	1,278	1,164	−114	17	All off feed in 13 days; 2 died (22d and 24th days); 1 refused to eat on 31st day.
411	3	Short-cooked, ether-extracted cottonseed meal.	733	945	+212	35	All died in 24–35 days.
408	3	Long-cooked cottonseed meal, No. 2.	1,133	1,197	+ 64	35	All sick in 35 days; 1 died on 38th day.

[a] Molasses fed with morning feed; collards and fresh greens for roughage.
[b] Average daily intake of food per rabbit was 10.6 gm.
[c] Very young rabbits from same litter.

TABLE II.—*Effects of various diets on the growth of rabbits*—Continued

Diet No.	Number of rabbits.	Food.	Average weight. Initial.	Final.	Change.	Percentage feed eaten is of initial weight.	Duration and result.
			Gm.	*Gm.*	*Gm.*		
409	3	"Buco" cottonseed feed.	1,515	1,648	+133	35	Discontinued after 37 days; 2 died in 40 and 44 days; 1 not affected and bore 5 young.
433	2	Long-cooked cottonseed meal, No. 3.[a]	1,333	1,175	−158	......	Off feed in one week; died in 26 days.
...	3	Long-cooked cottonseed meal, No. 2.[b]	1,637	1,507	−130	19	One died on the 19th day, 1 on the 22d day, 1 slightly off-feed in 27 days.
...	3	Ether-extracted cottonseed kernels.[b]	1,707	1,868	+161	27	Discontinued after 27 days. In good health.

[a] With dry alfalfa for roughage. [b] With fresh vetch for roughage.

With such a range of gradations and with the animals being distinctly affected in three to four weeks by even thoroughly cooked meals the results do not appear to us to be due to any lack of dietary essentials, especially in view of the very liberal feeding of fresh green food daily (collards, pea vines, vetch, clover, etc.).

Although there was never the slightest evidence that the molasses mixed with the cottonseed products for feeding had any bad effect, it was thought advisable to feed the meal in a different type of diet. Accordingly the animals were restricted to a monotonous dry diet containinf 40 per cent of alfalfa meal for roughage, 20 per cent of corn meal, 10 per cent of ground oats, and 30 per cent of the cottonseed product or protein concentrate to be compared (Table III). It was found that the same relations held true of cottonseed meal, except that the animals were thrown off their feed considerably quicker than on the regular experimental diet. The rabbits were not made sick by the ether-extracted kernels nor by soybean meal, but were quickly affected by the cottonseed-meal diets. The soybean meal was fed after the animals had been made sick on cottonseed-meal diets, and on this diet they regained normal appetite and weight. The long-cooked meal and the short-cooked meal were the same products as used in the rat, hen, and pig diets.

The great sensitiveness of the rabbit to cottonseed meal may be made clear by comparing the upper limit of toleration of the meal with the weight of meal which hens of similar weight withstood without being seriously affected. On various meals the rabbits sickened or died after

eating 17 to 35 per cent of their initial body weight when fed at the rate of 1 per cent or less per day, while hens withstood 600 to 700 per cent when fed at the rate of 1.25 to 1.6 per cent of body weight daily.

TABLE III.—*Effect of monotonous dry diets containing 30 per cent of concentrates on the growth of rabbits*

Period and rabbits No.	Feed.	Average weight. Initial.	Average weight. Final.	Change.	Duration and results.
		Gm.	*Gm.*	*Gm.*	
PERIOD 1:					
14, 16	Short-cooked cottonseed meal.	1,408	1,180	−228	Discontinued after 9 days. Both ate little at end. No. 14 became sick and died on 15th day.
9, 10, 11	....do	1,289	995	−284	Died in 16, 19, and 39 days. No. 9 gained at first.
29, 27	Long-cooked cottonseed meal 3.	1,254	1,095	−159	Discontinued after 15 days. Off feed.
13, 23	Long-cooked cottonseed meal 2.	1,873	1,505	−368	Do.
37, 38	....do	978	1,038	− 50	Discontinued after 16 days. One died, the other improved on this diet plus ferric ammonium citrate.
39, 40, 41	Ether-extracted cottonseed kernels.[a]	982	1,337	+355	Discontinued after 23 days. In good health.
PERIOD 2:[b]					
16, 13, 23, 27, 29	Soybean meal	1,298	1,599	+301	Discontinued after 19 days. All in good health.

[a] After 1 month on this diet the rabbits seemed rather tired of it, but had continued to gain and showed no sign of sickness.
[b] Period 2: the rabbits which were off feed on the cottonseed mixtures were changed to a similar mixture containing soybean meal.

EXPERIMENTS WITH POULTRY

In order to ascertain whether excessive amounts of cottonseed meal had any pronounced effect on fowls and also to study the reputed effect of the meal on the pigmentation of the yolk, several short experiments were conducted. The fact that the birds were alive and laying to some extent at the end of 170 days indicates the slight extent to which hens are affected by cottonseed meal. The meal used was made from kernels cooked for two hours under the same conditions those used in the long-cooked meals of the rat, rabbit, and pig experiments. Later, in one diet a short-cooked (28 minutes) meal was used. The composition of the diets is given in Table IV.

TABLE IV.—*Percentage composition of the poultry diets*

Feed.	Lot 1 (5 hens).	Lot 2 (5 hens).	Lot 3 (5 hens).	Lot 4 (5 hens).	Lot 5 (5 hens).	Lot 6 (6 hens).
Gossypol	......	......	......	......	0.2	......
Raw cottonseed kernels	......	......	......	......	......	30
Ether-extracted cottonseed kernels	......	......	......	40	......	......
Cottonseed meal, cooked two hours	40	30–0	30	......	......	......
Cottonseed meal, cooked 28 minutes	......	0–30	......	......	......	......
Meat meal	......	10	......	......	20–0	10
Corn meal	40–50	30	30	40–50	20–30	20
Wheat bran	......	10	20	......	30	10
Ground oats	......	10	20	......	19.8	20
Whole milk powder	20–10	......	......	20–10	10.20	10
Skim-milk powder	......	10	......	......	......	......
Protein (N × 6.25)	23.3	26.3	18.0	29.0	28.0	26.0
Protein from cottonseed	13.3	10.0	10.0	18.8	0.0	9.3
Cracked limestone	Ad libitum.					
Tap water	Ad libitum.					

The meat meal (80–85 per cent crude protein) was discontinued in lot 5 after 32 days, as the birds had declined greatly. Additional milk powder (10 per cent) and additional corn meal (10 per cent) were substituted in an attempt to make the diet more appetizing. This reduced the protein content to about 17 per cent.

Lot 6 was started after all in lot 5 had died.

Owing to inability to secure enough milk powder, after 140 days the amounts used in lots 1 and 4 were reduced to 10 per cent. Later, it was necessary to make use of skim-milk powder plus butter.

After the pig, rat, and rabbit experiments had shown considerable difference in toxicity between the long-cooked and short-cooked meals, the hens in lot 2 were fed on the short-cooked meal from the eighty-fifth day in place of the long-cooked meal.

The protein content of 40 per cent of extracted kernels is equal to about that of 55 per cent of cottonseed meal used in lots 1, 2, and 3.

The fowls were fed under ordinary conditions by an experienced poultryman. Each lot was confined in outdoor turf-covered pens about 6 by 30 feet. Covered sheds opening to the south were provided for roosting and shelter. The fowls were gradually worked up to the maximum amount of feed given each lot (0.9 pound) daily. Weighings were made every tenth day. The dead fowls were examined by Dr. B. F. Kaupp, Poultry Expert and Pathologist of this Station, under whose supervision the experiment was conducted. The results of this experiment are given in Table V.

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TABLE V.—*Effect of cottonseed products on the growth of hens*

Lot No.	Feed.	Number of birds.	Average age.	Average weight. Initial.	Average weight. Final.	Loss.	Period survivors fed.	Deaths and day of occurrence.
			Months.	*Pounds.*	*Pounds.*	*Per ct.*	*Days.*	
1	Long-cooked cottonseed meal, 40 per cent.	5	23	4.5	4.2	6.7	170	
2	Long-cooked cottonseed meal; short-cooked meal, 30 per cent.	5	25	4.4	4.2	4.5	170	
3	Long-cooked cottonseed meal, 30 per cent; no animal food.	5	24	3.9	3.0	23.1	170	2. 44th and 136th day.
4	Ether-extracted cottonseed kernels, 40 per cent.	5	31	4.7	3.8	19.1	170	2. 120th and 163d day.
5	Gossypol, 0.2 per cent.	5	17	4.3	2.8	35.0	......	5. 61st, 62d, 65th, 86th 91st day.
6	Uncooked cottonseed kernels, 30 per cent.	6	(?)	4.5	3.25	27.7	70	3. 28th, 55th, 70th day.

Lot 5 was fed in December, January, and February. Lot 6 was fed from March 10, when there was considerable grass, etc., in the yards. This enabled the animals to obtain food aside from the cottonseed mixture, and possibly explains why deaths were not so frequent as during a similar period in lot 5.

GENERAL EFFECT ON HEALTH OF HENS

Dr. Kaupp comments on the lots at the termination of the experiment as follows:

> Lots 5 and 6 suffered with pendulous crops, became sick of the feed, and all of lot 5 died. Lot 6 was following a similar route when discontinued. The birds in this lot were down in vitality.
>
> Lot 1 was in good physical condition except hen 8, which was suffering from pendulous crop. All the birds in lots 2 and 3 were in good physical condition. The birds of lot 4 suffered from pendulous crops, hen 19 being the worst. All the birds affected fully recovered, and were in good physical condition after a few days' feeding of normal rations for fowls.

There seems to be variance of opinion as to whether cottonseed meal is injurious to fowls. We may suggest that this is due to the use of different cottonseed meals. Thus, Hartwell and Lichtenthaeler (*3*), Clayton (*1*), and Osborne and Mendel (*7*) found no sign of toxicity. Kaupp (*4*), of this Station, has reported a high death rate among birds receiving large amounts of cottonseed meal, compared with control fowls fed on similar diets containing linseed meal.

The prompt effect of the diets of lots 5 and 6 on the health of the fowls indicates the highly toxic character of raw cottonseed and the gossypol therefrom. The effects of the other diet appeared after so long a period that possibly other factors may have been the cause of death in lots 3 and 4. But it will be noted that diet 3 was the poorest with respect to supplemental feeds of animal origin. Lot 4 received 40 per cent of ether-extracted cottonseed kernels, which is equivalent to about 55 per cent cottonseed meal, on the basis of the nitrogen content. This diet was therefore higher both in total protein and cottonseed protein. However, the fact that even this material has been found to be slightly toxic to rabbits and pigs would indicate that a toxic factor came into play. It was singular, however, that this lot was the only lot in which eggs were laid in the first part of the experiment. (See Table VI.)

To judge the results of the experiment by the effect on egg laying, the diet containing the ether-extracted cottonseed kernels, lot 4, was the least injurious of all during the first three months, while this relation was reversed with respect to cottonseed-meal lots during the last three months.

TABLE VI.—*Egg record (by months) of hens fed cottonseed products*

Lot No.	Food compared.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Total.
1	40 per cent cottonseed meal..	0	0	0	32	31	7	70
2	30 per cent cottonseed meal..	3	3	0	8	34	11	59
3	.....do......................	0	0	0	6	25	0	31
4	40 per cent extracted cottonseed kernels..............	13	17	29	0	2	0	61
5	0.2 per cent gossypol........	0	0	0	......	......	......	0
6	30 per cent raw cottonseed kernels..................	......	......	......	1	0	10	11

Another noteworthy feature, indicating the high resistance of fowls, was the fact that the short-cooked meal, which was found distinctly more injurious to rabbits and swine, had no more effect on the fowls than the long-cooked meal.

AMOUNT OF FEED EATEN BY EACH LOT

The birds in these experiments were accustomed gradually to the diet. The amounts fed were gradually increased to a maximum of 0.9 pound (408 gm.) per lot per day of feed 81.7 gm. per bird containing from 24 to 32 gm. of cottonseed product. The diets of lots 5 and 6 were but poorly consumed, and there was consequently a steady loss in weight. The other feeds were eaten regularly, although at the end the birds in lot 4 were losing appetite. The record of the feed eaten is given in Table VII.

TABLE VII.—*Feed consumption of the six lots of hens fed cottonseed products*

Lot No.	Food compared.	Total feed consumed.		
		Per lot.	Per bird.	Cottonseed product.
		Pounds.	*Pounds.*	*Pounds.*
1	40 per cent cottonseed meal	148.7	29.7	11.9
2	30 per cent cottonseed meal	152.5	30.5	9.2
3	.....do	147.7	29.5	8.9
4	40 per cent extracted cottonseed kernels	132.6	26.1	[a] 10.6
5	0.2 per cent gossypol	29.5	5.8	[b] .0117
6	30 per cent raw cottonseed kernels	28.6	4.8	1.43

[a] This is equivalent to 14.6 pounds of cottonseed meal on basis of nitrogen content.
[b] This amount of gossypol (5.3 gm.) is equivalent to 1.75 pounds of raw cottonseed kernels.

EFFECT ON COLORATION OF THE YOLK

It has been noted frequently that eggs from hens fed cottonseed meal show a peculiar brownish discoloration of the yolk, giving them the appearance of very old eggs. Several eggs from each of the cottonseed-meal lots were examined, but seemed to be of normal appearance. There were no eggs laid by lot 5 (fed gossypol). Possibly, if a diet of intermediate toxicity between the diet of lot 5 and the diets of lots 1, 2, 3, and 4 had been fed—that is, so that laying was not entirely prevented—there would have been some abnormal appearance in the eggs. Out of 10 eggs examined in the lot fed raw cottonseed kernels the yolks of 4 were very perceptibly affected with the brown discoloration. The other yolks were affected but slightly, or not at all. The data are rather meager, but at least indicate that, when this phenomenon occurs in eggs, it may be due to gossypol which is unchanged, owing to insufficient cooking of the cottonseed. Whether this phenomenon is due to the peculiar pigments of the cottonseed or to some physiological action of gossypol on the fowl has not been ascertained. The authors are of the opinion that the discoloration is not due to the deposition of gossypol or related substance in the yolk, such as occurs with carotin and xanthophyll. This opinion is based on the fact that, although gossypol is a fat-soluble pigment, it readily forms salts which are not fat-soluble and is also so easily oxidized that one would not expect it to be stored as such in egg fat. Thus, it does not occur in milk fat or body fat of animals fed with cottonseed meal. In fact, Palmer and Eccles (*9*) have shown that cottonseed meal tends to produce a colorless butter, owing to the very small amounts of carotin and xanthophyll present.

EXPERIMENTS WITH SWINE

As a result of our preliminary experiments (*16*) with rats, rabbits, and pigs, in which it was shown that raw cottonseed and gossypol were highly toxic, it appeared that the cooked kernels, and therefore cottonseed meal, were decidedly less toxic by reason of some transformation of

gossypol. At the same time it appeared that cottonseed meal has nutritive limitations to which others have attributed the phenomenon of cottonseed-meal "injury." Such limitations are, of course, not peculiar to cottonseed, but exist in all seeds. Thus, corn, wheat, rice, oats, etc., have been shown by McCollum and associates to possess an insufficient supply of the fat-soluble growth-promoting substance. As a single source of protein, minerals, and vitamines for rats cottonseed meal seems far superior to any of these grains. In fact, cottonseed meal has an ash content about seven times greater than that of corn.

Hart, Miller, and McCollum (2) have shown that the wheat embryo contains a toxic material which is manifest in certain limited diets, but the effect of which is overcome in a highly efficient diet.

Recognizing the profound influence that cooking cottonseed meal has on the toxicity of the raw seed, we chose for our experiments two meals the preparation of which has been previously described in this article.

The objects of the pig experiments were to ascertain—

(1) Whether "injury" similar to cottonseed-meal "injury" was manifested in diets containing high protein concentrates whose nutritive properties we might expect to be similar to cottonseed meal.

(2) Whether a meal which had been subjected to cooking for a period which represents the upper limit in cottonseed milling would prove definitely injurious to swine.

(3) Whether, as we predicted, a short-cooked meal would prove more active than a long-cooked meal, other things being equal.

(4) Whether by improvement of the diet (diet 6) by the addition of of butter fat, meat scrap, and the inorganic elements (calcium, sodium, chlorin) which are the least abundant of the necessary elements in the meal, or by addition of milk products (diet 7), which would improve the protein, mineral, and vitamine content of the diet, cottonseed meal poisoning might be averted.

TABLE VIII.—*Percentage composition of the swine diets*

Feed.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 5.	Lot 6.	Lot 7.
Cottonseed meal 1, long-cooked (2 hours)	25					30	30
Cottonseed meal 2, short-cooked (28 minutes)		25					
Soybean meal			25				
Peanut meal				25			
Ether-extracted cottonseed kernels					25		
Cracked corn	65	65	65	65	65	42	40
Wheat bran	10	10	10	10	10	20	20
Milk solids, skim milk, or buttermilk							10
Meat scrap						3	
Butter						5	
Salt mixture						14	
Approximate protein content (N × 6.25)	16.3	16.8	19.5	16.0	19.7	18.7	20.5

(5) The object of feeding cottonseed kernels (diet 5) was for comparison with what might be expected of cottonseed meal in view of its protein and ash content. This diet is practically identical with the diets of lots 1 and 2, except for the toxic factor, which is greatly diminished but probably not entirely removed by extraction with ether.

We may also call attention to the fact that this diet contained, on a basis of nitrogen content, the equivalent of approximately 35 per cent of cottonseed meal.

Seven lots of three pigs each were fed in pens bedded with wood shavings. The pigs were Berkshires about 6 months old.

The composition of the diets is given in Table VIII.

The salt mixture supplied to lot 6 consisted of 350 gm. of calcium lactate (dry basis), 100 gm. of sodium chlorid, and 30 gm. of ferric ammonium citrate to each 100 pounds (45.35 kilos) of feed.

The pigs were fed on diminished rations at first. The amounts fed were gradually increased until the pigs received all they would eat readily twice daily in the form of slop. The animals were supplied with city tap water.

On the sixth day of the experiment it was necessary to cut down the ration of the pigs in lots 1 and 2. And from this time on, these pigs showed increasing dislike for their feed. Frequently at this time pigs in lot 2 (short-cooked meal) were observed by the feeder to vomit after eating. Finally, lots 1 and 2 were consuming only 5 pounds of feed per day as compared with 8 to 10 pounds for the other lots. This behavior was naturally reflected in the weight records. The animals in the other cottonseed-meal lots maintained excellent appetittes and showed good gains for a much longer period.

Table IX gives the data on the feeds consumed by the various lots.

TABLE IX.—*Feed consumption (in pounds) by swine fed cottonseed products*

Period.	Cottonseed meal 1 long-cooked (lot 1).	Cottonseed meal 2 short-cooked (lot 2).	Soybean meal (lot 3).	Peanut meal (lot 4).	Ether-extracted cottonseed kernels (lot 5).	Long-cooked cottonseed meal, supplemented. Lot 6.	Lot 7.
1–20 days	136.50	135.50	148.00	148.00	148.00	148.00	148.00
21–40 days	149.50	116.50	214.00	212.00	168.50	208.50	205.50
41–56 days	78.00	76.50	157.50	157.50	139.50	148.50	141.00
41–60 days	....	....	201.50	201.50	178.50	178.00	167.00
61–83 days	....	....	276.00	276.00	228.00	103.90	4.00
Total feed	[a]364.00	[a]328.50	[b]839.50	837.50	723.00	638.90	524.50
Total protein concentrates	91.30	82.10	209.90	209.40	180.80	191.70	178.20
Average per pig	30.30	27.40	69.90	69.80	60.30	63.90	52.50
Average food per day for first 56 days	6.39	5.80	9.31	9.28	8.51	9.37	8.83

[a] Amount offered. Lots 1 and 2 frequently left much of their food.

[b] The smallest pig in the soybean lot was slaughtered on the seventieth day, following an accident by which its backbone was broken at the eleventh dorsal vertebra. For convenience, it is assumed that in lot 3 three pigs were continued at the same rate to the end of experiment.

The pigs fed cottonseed meal received the feed up to the time definite symptoms appeared, and until it was evident that the survivors would not live if continued on the diet. The first death occurred in lot 1 on the forty-seventh day, No. 3, followed shortly by two deaths in lot 2, No. 4, 5 (fig. 4). The survivors in lots 1 and 2 were then eating very poorly and were more or less sick, so that these feeds were terminated on the fifty-sixth day.

The gains made in each lot per pig are given in Table X.

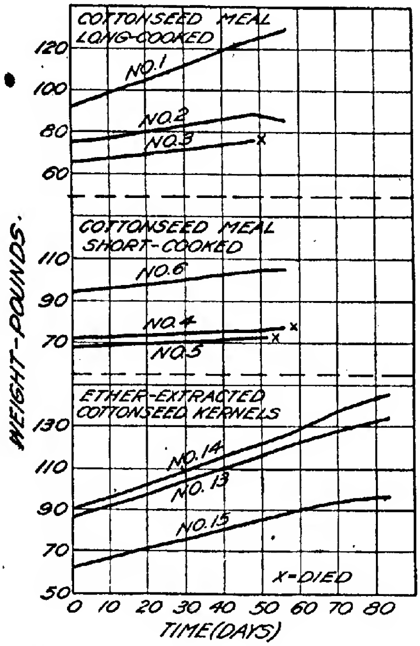

FIG. 4.—Graphs showing the effect of cottonseed products on the growth of pigs. The toxicity of long-and short-cooked meals is quite similar, the pigs showing a gain in weight for a time, but they were either sick or dead in 56 days. The pigs fed on ether-extracted cottonseed kernels continued to thrive throughout the entire period, proving conclusively the removal of the toxic substance by the ether.

The claim that cottonseed meal "injury," or so-called poisoning, in pigs, is caused by dietary deficiencies or by erroneous practices in feeding seems to be without weight, in view of the comparatively excellent results obtained with pig diets containing ether-extracted cottonseed kernels. Comparing the chemical composition of diets containing corn and cottonseed meal with one containing corn and ether-extracted cottonseed kernels, we see that the protein and mineral factors are kept identical. Possible objection might be raised that the vitamines are either destroyed or pressed out into the oil in the manufacture of the meal. This does not seem to be the case. The temperatures reached by the seed in the manufacture of cottonseed meal are very little if any over 100° C., although the material is in a container whose walls are much hotter than this. This higher temperature of the walls of the cooking drums is maintained by steam under 20 to 50 pounds' pressure. Temperatures taken of material fresh from the drums show apparently a constant limit of 100°, owing to the fact that the kernels are moist, partly from their own or added moisture, which evaporates and thus keeps the temperature from rising.

TABLE X.—*Effect of various rations on the growth of pigs*

Ration.	Average daily gain per pig. 48 days.	56 days.	70 days.	83 days.	Percentage gain in 56 days is to initial weight.	Total gain in 48 days.	Feed eaten. Total.	Per pound of gain.	Number of deaths.	Day on which death occurred.
	Pound.	*Pound.*	*Pound.*	*Pound.*		*Lbs.*	*Lbs.*			
Cottonseed meal 1, long-cooked (lot 1)	0.368	0.335	......	......	21.7	53	327	6.1	1	47
Cottonseed meal 2, short-cooked (lot 2)	.167	.170	......	......	11.9	24	291.	12.1	2	52, 56
Soy-bean meal (lot 3)	.646	.678	0.722	0.70	50	93	431	4.6	0	......
Peanut meal (lot 4)	.555	.588	.633	.655	47	80	431	5.4	0	......
Ether-extracted cottonseed kernels (lot 5)	.583	.583	.571	.55	41.4	84	380	4.5	0	......
Cottonseed meal 1, butter, salts, meat scrap (lot 6)	.701	.702	......	......	48.7	101	417	4.1	2	56, 75
Cottonseed meal 1, milk powder (lot 7)	.799	.782	......	......	53.9	115	419	3.6	2	52, 59

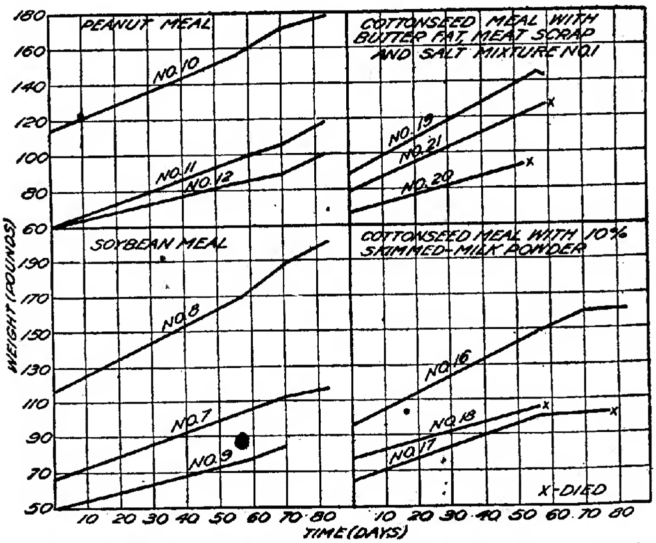

FIG. 5.—Graphs showing the effect of various diets on the growth of pigs. Pigs fed on peanut meal and soybean meal show a decided and regular gain in weight, while those fed with cottonseed meal supplemented by other feed made good gains only during the early part of the feeding period. The toxicity of the meal is shown by the death of four of the pigs and the sickness of the others before the end of the time.

Whether the fat-soluble vitamines pass into the crude oil to any extent is not known. McCollum (*5, 6*) has shown that ether extraction of many seeds does not remove it, possibly as it is a combination not

soluble in ether. Probably the same holds true of oil extraction. McCollum has found that the refined oil lacks the growth-promoting constituent but the refined oil has been treated in ways which might remove this constituent. Richardson and Green (*12*) state that the ether extract of cottonseed flour contains this fat-soluble growth-promoting factor.

TABLE XI.—*Weight record of pigs on various diets*

Lot No.	Feed compared.	Animal No.	Weight. Initial.	Weight. Final.	Weight. Gain.	Gain.	Remarks.
			Lbs.	*Lbs.*	*Lbs.*	*Per ct.*	
1	Cottonseed meal, long-cooked.	1	92.0	128.0	36.0	......	Sick 56th day.
		2	75.0	86.5	11.5	......	Do.
		3	66.0	76.0	10.0	......	Died 47th day.
	Average	....	77.7	96.8	19.2	24.7	
2	Cottonseed meal, short-cooked.	4	72.0	76.5	4.5	......	Died 56th day.
		5	68.0	73.0	5.0	......	Died 52d day.
		6	93.0	105.5	12.5	......	Sick 56th day.
	Average	....	77.7	85.0	7.3	9.4	
3	Soybean meal	7	67.0	118.0	51.0	......	
		8	117.0	200.5	83.5	......	
		9	50.0	83.5	33.5	......	Slaughtered.
	Average	....	78.0	134.0	56.0	71.8	
4	Peanut meal	10	114.0	179.0	65.0	......	
		11	60.0	118.0	58.0	......	
		12	60.0	100.0	40.0	......	
	Average	....	78.0	132.3	54.3	69.6	
5	Ether-extracted cottonseed kernels.	13	86.0	133.0	47.0	......	
		14	90.0	146.0	56.0	......	
		15	62.0	96.0	34.0	......	
	Average	....	78.7	125.0	45.7	58.0	
6	Cottonseed meal, long-cooked, butter, meat scrap, and salt mixture.	16	96.0	161.0	65.0	......	Sick 75th day.
		17	65.0	102.0	37.0	......	Died 76th day.
		18	77.0	106.0	29.0	......	Died 56th day.
	Average	....	78.7	123.0	43.7	55.5	
7	Cottonseed meal, long-cooked, plus 10 per cent milk powder.	19	88.0	143.0	55.0	......	Sick 62d day.
		20	68.0	93.0	39.0	......	Died 52d day.
		21	79.0	127.0	48.0	......	Died 59th day.
	Average	....	78.3	121.0	44.0	56.2	

The chief chemical difference between cottonseed meal and ether-extracted cottonseed kernels seems to be, then, the presence of gossypol, or the decomposition products of gossypol, to which cottonseed-meal poisoning in swine appears to be due.

It will be noted (fig. 5) that pigs receiving the meal in the supplemented diets ate much more meal and made the best average gains.

With one exception they lived considerably longer than the pigs on unsupplemented diets. None of the pigs which died from the effects of the meal were in poor nutrition; while the pigs of lots 6 and 7, at death, were in normal nutrition. (Table XI.) If we accept the criterion suggested by Wells and Ewing (*14, p. 22*), that—

if well nourished animals die of the injury as is claimed the same cannot be due to a deficient diet and inanition, but must be due to a toxic effect,

then the results of these pig experiments should render nonvalid their conclusion that—

Cottonseed meal injury is due in large part to inadequate diets.

It is doubtful, however, whether this criterion may be generally applicable to deficiency diseases—for example, to beriberi. While the growth curves for small experimental animals, especially fowls and rats, on deficient diets show steep downward slopes to absolute failure, the authors are not aware of cases analogous to those which we report with swine on cottonseed-meal diets (lots 6, 7, Table XI)—namely, the sudden death while gaining rapidly in weight and while in normal control of the limbs. (See also fig. 5, No. 16–21.)

The hypothesis proposed by Rommel and Vedder that cottonseed-meal poisoning in pigs is beriberi, caused by a deficiency in the ration, can not be supported in the terms of our present day ideas of the requirements of an adequate diet. For example, it is evident that our diet 6 is well supplied with the water-soluble and the fat-soluble vitamines, the former having been shown to occur in abundance in cottonseed meal and in corn, while the only moderate deficiency of the fat-soluble accessory in these seeds is overcome by the addition of 5 per cent butter to the diet. The addition of a salt mixture containing calcium, sodium, and chlorin, to supplement these least abundant of the necessary mineral elements in the seeds of plants eliminates the possibility of a deficiency of mineral (matter) as the cause of failure.

As stated before, diets 1 and 2 were discontinued after deaths began to occur in these lots and when the surviving pigs were so noticeably sick that death seemed imminent.

Of the two pigs which survived in lots 6 and 7, one lost in weight and the other made no further gain after discontinuance.

Post-mortem examination of all the pigs which died from cottonseed "injury" showed uniformly the characteristic symptoms associated with the disease—viz, congestion and edema of the lungs, hydrothorax, hydropericardium, etc. The pigs which were slaughtered at the close of the 83 days' experiment were in good condition, and no pathological lesions were evident.

AN EXPERIMENT WITH COMMERCIAL COLD-PRESSED MEAL

Experiments with rats and rabbits revealed the fact that cold-pressed meals were not so markedly toxic as certain hot-pressed meals made from very dry seed and insufficiently cooked. It was thought desirable to ascertain how a cold-pressed meal would affect pigs.

Two pigs in pens were fed for 30 days on a cold-pressed meal and corn meal (1:2). The pigs were fed as much as they would readily eat. They consumed relatively much more meal than was eaten during the previous experiments with hot-pressed meal, and maintained good appetites throughout. No safe conclusion may be drawn, but it was evident that these animals were less affected in this short period than the pigs on diets 1 and 2 of the previous experiments in a similar period. The fact that by far the greater part of the gossypol of the raw seed is removed from cold-pressed cottonseed meal and that the remaining small amount may undergo oxidation, etc., while the hot meal is exposed to the air should prove of interest in the practical matter of avoiding cottonseed-meal "injury" of swine. The results of this experiment are given in Table XII.

TABLE XII.—*Effect of commercial cold-pressed cottonseed meal on the growth of pigs*

Pig No.	Weight.			Average quantity of cottonseed meal eaten.	
	Initial.	Final.	Gain.	Total.	Per day.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
22	70	85	15	32.1	1.07
23	85	99	14	32.1	1.07

GENERAL SUMMARY AND CONCLUSIONS

Various cottonseed products, including raw cottonseed kernels, ether-extracted kernels, gossypol, and several meals, have been fed to rats, rabbits, poultry, and swine.

Raw cottonseed kernels and the gossypol therefrom have been found highly toxic to all these animals. Cooking the kernels under oil-mill conditions causes a profound reduction in toxicity. This change is so great that the thoroughly cooked products show no pronounced toxic effect on rats and poultry in suitable diets. Thoroughly cooked meals, however, appear to be definitely injurious to rabbits and swine, which are peculiarly susceptible to cottonseed-meal "injury." Rats and fowls are able to withstand much larger relative amounts of cottonseed meal for longer periods. In the "cold-pressing" process of making cottonseed meal the toxic substance passes into the oil to a great extent, thus leaving a meal which may be less harmful than certain hot-pressed meals.

RATS

Cottonseed meal, cottonseed flour, and ether-extracted raw cottonseed kernels have been fed to rats under comparable conditions. Rats fed on extracted kernels have shown superior growth over those on cottonseed meal or cottonseed flour. From this fact it is inferred that even in well-cooked products there remains something slightly deleterious to rats fed on diets containing these as the sole source of vitamines, protein, and minerals. Diets containing well-cooked cottonseed products, with a small amount of milk powder, appear to be as efficient for rats as the control milk diet.

The degree of toxicity of cottonseed meals depends on the thoroughness of cooking in the oil mill. This change appears to be due to oxidation of the gossypol to a substance which we have called "D-gossypol." Some meals may be much more toxic than others, through failure to complete this change. Since evidence shows that the gossypol of the raw seed may be entirely changed to this far less toxic material, it is suggested that the highly toxic effect of the raw cottonseed be described as cottonseed poisoning and that injury due to the meal be described as cottonseed-meal poisoning or injury.

Diets containing cottonseed meal with corn meal, or soybean meal with corn meal, as the sole source of nutriment have led to failure of our rats. The addition of calcium lactate, sodium chlorid, and butter tends to avert this failure.

RABBITS

Rabbits are much more susceptible than rats to cottonseed-meal poisoning. They have been very quickly affected by much smaller relative amounts of the meal in diets which are apparently adequate for these animals.

POULTRY

Aside from an apparently diminished egg production, excessive amounts of cottonseed meal have not appeared to be very injurious to hens. Some evidence is presented to show that the presence of unchanged gossypol in the diet may cause a peculiar discoloration of the egg yolk.

PIGS

Pigs have been fed on diets designed to compare the effect of cottonseed meals with similar protein concentrates, such as peanut meal soybean meal, and ether-extracted cottonseed kernels.

Unsuccessful attempts have been made to avert cottonseed-meal "injury" by supplementing a thoroughly cooked meal with (a) meat scrap, calcium lactate, sodium chlorid, and butter fat, or (b) 10 per cent of skim-milk powder.

Cottonseed meal exerts on pigs a harmful effect, which is not averted by improving the diet with efficient food materials. Such a harmful effect is not produced by similar feedstuffs. Hence, we conclude that the cottonseed-meal "injury" of swine is due, not to deficient diets, but to the presence of a toxic substance. In our opinion this toxic substance in cottonseed meal is the derivative of gossypol which we have called "D-gossypol."

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